THE UNIVERSITY OF ALBERTA

BOVINE SERUM TRANSFERRINS - INHERITANCE AND RELATION TO PRODUCTION TRAITS

A THESIS

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GRAHAM ALEXANDER EDWARD GALL

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ABSTRACT

An investigation was undertaken to determine gene frequencies at the transferrin locus and to determine the relation of the transferrin genotypes to efficiency and productivity for the cattle maintained in the University of Alberta beef breeding project.

The characteristics of the transferrin patterns observed, although in general agreement with the three allelic system postulated for the control of the variation, suggested that a simple one gene - three protein relation was not sufficient to account for all the variation present in some transferrin patterns.

Three transferrin types were observed in the Hereford herd and six in the hybrid herd. The gene frequencies found in the two herds, calculated using the three allele hypothesis established by Ashton (1958b) were Tf^{A} = 0.47 and Tf^{D} = 0.53 for the Hereford herd and Tf^{A} = 0.38, Tf^{D} = 0.51 and Tf^{E} = 0.11 for the hybrid herd. Great variation in gene frequency also existed between the Angus, Galloway and Charolais breeds in the hybrid herd.

Parentage data were in general agreement with the theory of inheritance although the data indicated that offspring in the hybrid herd carrying the Tf^{E} allele occurred at a lower level than expected. There was also an indication of a heterozygous advantage for the Tf^{A}/Tf^{D} genotype.

The frequency of occurrence of the Tf^{E} allele in the progeny genotype in the hybrid herd suggested that the Tf^{E} allele was at a selective disadvantage, prepartum, in the herd examined at least when the Tf^{E} allele was present in the sire's genotype only.

The effects of sire, breed group of dam, sex of calf and transferrin genotype on birth weight and calculated 180-day weight of calf

were studied for the two calf crops, 1961 and 1962. Although breed group and sire differences were found, no relation was observed between the traits studied and transferrin genotype. The analysis showed an indication that the Tf^A/Tf^D heterozygote may be superior in some traits but the differences were not consistent. It is suggested that any differences that may exist between the transferrin genotypes would be small and since the sources of variation in the herds studied were numerous, it is probable the numbers available were not sufficient to detect small differences.

Differences due to effects of breed group and pregnancy were found among the performance of cows during the winter of 1961-62, as estimated by accumulated weight loss. Pregnancy was found to reduce weight loss during the winter and calving and nursing were found to inhibit weight gain in the spring as compared to contemporary open cows. No statistical relation was found between transferrin type and accumulated weight loss of the cows, although Tf^A/Tf^D genotypic individuals appeared to have a selective advantage over some other genotypes.

Standard cow weight, as estimated by the average of a post-weaning weight and a post-calving weight, was not significantly ($P > 0.05$) influenced by transferrin genotype, however the least squares estimates for this trait in the hybrid herd suggested that Tf^A/Tf^D genotypic individuals were heavier than individuals of the other genotypes. Standard weight of the Hereford cows was influenced by pregnancy, open cows being 45 lb. heavier than pregnant cows.

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INTRODUCTION

The general adherence to pure breeding practices during many years of economic improvement of livestock has resulted in the fixation of many known genes controlling simply inherited traits. Furthermore, it is generally assumed that many polygenic systems influencing economically important traits have become fixed to a large extent in these improved populations. Since improvement in livestock through breeding and selection is dependent on the genetic variation present in the population, the breeder and geneticist are interested in following any group of genes segregating in the population.

The extension of biochemical and immunological techniques into the genetics field has given the geneticist a tool with which to detect individual genes segregating in livestock populations. It has also brought the geneticist a step closer to an understanding of gene action and of basic physiological processes. Proteins were recognized as fundamental elements in these processes, with the result that a number of polymorphic biochemical systems with simple inheritance have been detected in farm animals during recent years. Of these, the genetic systems involving blood group antigens in poultry and cattle have received extensive study. More recently, the development of a method of zone electrophoresis in starch gel has made possible the elucidation of another polymorphism in cattle, which involves the beta-globulin fraction of blood serum proteins.

The ability to identify such genes segregating in a population may be of help to the breeder in several ways. First, the genes themselves may influence the economic performance of the animal. Second, linkage of these identifiable genes with other genes which influence economic

performance, but which cannot be individually distinguished, provides a possible identifying marker for lines capable of maximum combining ability. Third, since several alleles are generally present in a population, they can provide a valuable check on the validity of pedigrees, and similarly can be used in the determination of twin zygosity. Fourth, extensive study of the end products of the gene observed in the phenotypes, makes possible the study of gene action. With these possible uses of known genetic systems, the study reported herein was undertaken specifically to determine the population structure of the transferrin locus in the beef cattle maintained at the University of Alberta Ranch and to study the relation of the transferrin genotypes to production traits.

LITERATURE REVIEW

I. Historical Aspect of Electrophoretic Methods

Serum proteins have been classified by methods based on differential solubility into albumins, euglobulins, and pseudoglobulins (Cantarow and Schepartz, 1957). Each protein class consists of several to many different proteins of similar solubility. The development of moving-boundary electrophoresis, also called the Tiselius method, made possible a further protein classification based on electrophoretic mobilities in an electric field. The fractions so obtained were designated albumin, and alpha, beta and gamma globulin in order of their decreasing mobilities. Because the proteins in solution in the moving-boundary method overlap each other, actual physical separation of the proteins is not possible. Zone electrophoresis, an extension of the techniques of paper chromotography, has proven of value in obtaining electrophoretically discrete proteins. Other advantages of the zone method over moving-boundary include its adaptability to small quantities of material and freedom from quantitatively important boundary anomalies (Smithies, 1955).

II. Starch Gel Electrophoresis of Serum Proteins

A. Techniques

The two methods of zone electrophoresis generally used, the filter-paper method of Kunkel and Tiselius (1952) and the starch-grain method of Kunkel and Slater (1952), do not have a resolving power as great as that of the moving-boundary method. The resolving power of the filter-paper method is inhibited by the high adsorptive effect of the paper and by the difficulty of not being able to put the sample on the paper in a sufficiently narrow zone. The serial analysis necessary with the starch-grain

method limits the resolving power of this method because of difficulty in obtaining thin serial sections (Smithies, 1955).

In an attempt to overcome the limitations of the filter-paper and starch-grain methods, Smithies (1955) developed a method of zone electrophoresis which involved the use of a starch gel as the supporting medium. The resolving power of the starch gel electrophoretic method is often superior to that of the classical Tiselius method while giving the advantages of low absorption, characteristic of the starch-grain method and desirable staining properties, characteristic of the filter-paper method. The technique is also adaptable to very small quantities which can be inserted in a sufficiently narrow band for convenient protein detection.

Smithies (1959a) modified the procedure by placing the starch gels in a vertical position during electrophoresis allowing the proteins to migrate downward as opposed to horizontally in the original procedure. The vertical modification permitted the introduction of the sample into the gel without the aid of a supporting material resulting in increased resolution and superior repeatability of protein separation.

B. Theoretical Considerations

In any form of electrophoresis, the effective force applied to an ion is directly proportional to the net charge carried by the ion. Smithies (1959b) discusses the influence which the isoelectric point, the cationic and uncharged acid make up, and the ion binding properties of a protein may have on the net charge carried by a protein. He concluded that the charge carried by a protein in a buffered solution is a function of the nature of the protein and of the composition and pH of the buffer solution.

The greater resolving power of the starch gel method over other types of zone electrophoresis is chiefly a consequence of the use of a supporting medium of a pore size which approaches the molecular size of serum protein molecules. Smithies (1955) observed that proteins of similar mobilities in moving-boundary and filter-paper electrophoresis may have widely differing mobilities in starch gel. Smithies (1959b) was able to support the hypothesis that molecular size plays an important role in determining the mobilities of proteins in starch gels. He observed that serum B-lipoprotein, serum transferrin, and free hemoglobin migrate in starch gel in the reverse order of their molecular sizes, despite the fact that their mobilities are approximately the same on filter paper.

Because of the molecular dimension selectivity of starch gel, the utilization of the method is limited to proteins of a size not larger than the useful dimensions of the gel pores. Smithies (1955) found that much of the γ-globulin in blood serum did not enter the gel, but remained in the sample slot.

Recent detailed work with rat blood serum by Espinosa (1961) has borne out the above theoretical considerations involved in starch gel electrophoresis.

C. Results with Serum Proteins

Smithies (1955) described the electrophoretic pattern obtained with a typical serum from each of three serum groups for normal adult humans (Fig. 1). The pattern of type I serum appeared similar to that obtained by conventional methods with respect to the main components, namely albumin and alpha, beta and gamma globulins. However, additional bands were observed particularly in the IIA and IIB patterns.

In an attempt to identify, in the classical terms, the additional bands observed in starch gel, Smithies (1955) and Poulik (as cited by

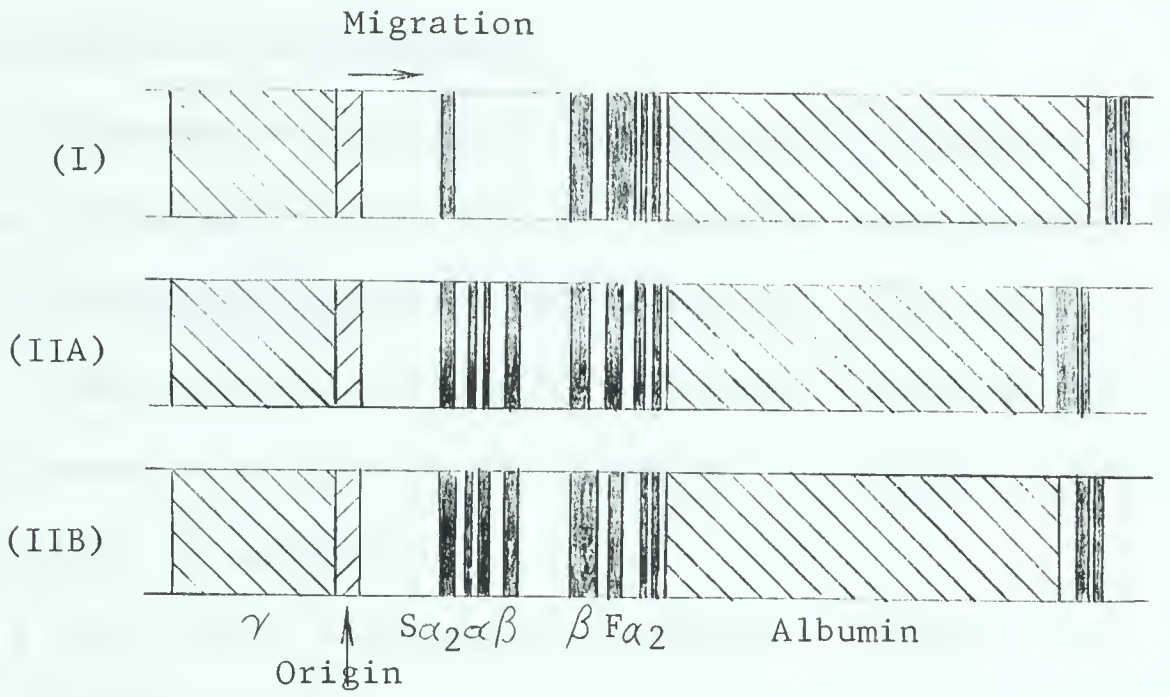

Fig. 1. Schematic representation of the three serum protein patterns described by Smithies (1955) illustrating the nomenclature use to designate the components.

Smithies, 1955) devised a procedure in which the bands observed on starch gel were compared to the classical bands obtained on filter paper. The new components demonstrated by the starch gel method were found to be derived from the α-globulin complex. The components were identified by naming the bands in terms of their position relative to the major components. No relation other than position was implied. A summary of the system of classification outlined by Smithies (1955) follows:

1. Albumin was identified from a quantitative consideration.

2. The protein zone migrating in the retrograde direction was identified as γ-globulin in nature.

3. The intermediately migrating band common to all three serum types was proven to be β-globulin.

4. The two components migrating more rapidly than the broad albumin zone were referred to as pre-albumins 1 and 2, 1 indicating the faster moving component.

5. The faint components migrating somewhat slower than albumin were referred to as post-albumins.

6. The more distinct zone migrating just behind the post-albumins, but slightly ahead of the β-globulin zone proved to be part of the α_2-globulin complex and was referred to as F (fast) α_2-globulin.

7. The darkly staining zone migrating slower than the β-globulins was also proven to be part of the classical α_2 complex and was referred to as S (slow) α_2-globulin.

8. The faintly staining zones migrating between the S α_2-globulins and the β-globulins were referred to by Smithies as $\alpha\beta$-globulins.

Poulik (cited by Smithies 1959b) and Smithies (1958) confirmed the identification of the components by a two-dimensional electrophoretic system involving a combination of the filter-paper and starch gel methods. The serum samples were first fractionated on filter-paper followed by a fractionation in a second dimension in starch gel, by placing the complete, moist filter paper strip in a starch gel. This method also shed some light on the nature of some of the zones. The $\alpha\beta$-globulins of Smithies (1955) were found to be part of the classical α_1-globulin complex. The slower starch gel pre-albumin$_2$ zone was found to be α_1-globulin in nature and was established as an acidic α_1- glycoprotein by its electrophoretic and immunological properties. In the same respect S α_2-globulin was established as a high molecular weight α_2-glycoprotein (Poulik, 1959, as cited by Smithies, 1959b).

Poulik (Smithies, 1959b) and Giblett, et al. (1959) compared the electrophoretic properties of the chief β-globulin present in most blood serum samples with those of purified transferrins, the iron binding proteins

of the blood, and found the two to be identical. Espinosa (1961) observed similar results using rat serum. He demonstrated the presence of at least ten α_1-globulin fractions most of which were masked by the other more distinct bands. Similar results were also obtained by Ashton (1957b) and Smithies (1958) with cattle serum, by Kristjansson (1960a and b) with swine serum, and by Beaton, et al. (1961) with rat serum.

III. Genetic Variation in Serum Proteins

The first recognized genetic variation involving relatively specific proteins of the serum of animals was described by Smithies (1955). He observed three general protein pattern types, two of which were more alike than the third (Fig. 1). The results of family studies indicated that the differences were under simple genetic control (Smithies and Walker, 1955) postulated to implicate a single autosomal locus, occupied by either of a pair of alleles with no dominance exhibited. Further work established that the sera of the three groups differed in proteins with the characteristic property of binding hemoglobin and with a migration pattern in the α_2-globulin zone. Smithies and Walker (1956) suggested that these serum types be referred to as haptoglobin types, and that the genetic locus involved be designated the haptoglobin locus by the symbol Hp.

Ashton (1957a) extended the method of analysis to other mammalian sera including the cow, dog, pig and horse. Ashton (1957b) and Hickman and Smithies (1957) described the occurrence of variation in the serum β-globulins of cattle (see section IV of this review).

A polymorphic system was detected in cattle by Ashton (1958a) involving a protein which he designated "thread protein". Four phenotypes were described, depending upon the presence or absence of one or both of two thread like zones. In starch gel the two zones migrate in the region

of $S\alpha_2$-globulin, one more slowly and the other more rapidly than the $S\alpha_2$ band. The postulated genetic mechanism controlling this polymorphic system involves three alleles of an autosomal locus. Ashton had difficulty distinguishing between phenotypes exhibiting both "thread protein" zones and those in which neither zone is present.

A β-globulin polymorphism similar to that found in cattle has been observed in human sera (Smithies, 1958). Horsfall and Smithies (1958) established that the β-globulin differences observed were under the genetic control of a pair of autosomal alleles with no dominance. Further human β-globulin patterns have been observed by Smithies (1958), Harris et al. (1958), and Smithies and Hiller (1959). Giblett et al. (1959) demonstrated the β-globulins exhibiting the polymorphic system to be transferrins.

Ashton and coworkers reported observing genetic variation involving the β-globulins of several mammalian species and postulated the genetic mechanisms controlling the variations; a two allele system for goats, based on three observed phenotypes (Ashton and McDougall, 1958); a five allele system for sheep, to explain the fourteen observed phenotypes believed to represent the ten heterozygous forms and four of the possible five homozygous forms (Ashton, 1958 c and d); three β-globulin phenotypes in swine controlled by a pair of autosomal alleles, as well as three observed "thread protein" phenotypes similar to that observed in cattle by the same worker (Ashton 1960a); and a two allele, three phenotype genetic system for the control of β-globulin variation in the serum of mice (Ashton and Braden, 1961).

Kristjansson (1960a) described a polymorphism observed in one of the blood serum proteins in swine, designated protein B, and suggested

that the variation was controlled by a single pair of alleles exhibiting partial dominance. By means of a two dimensional starch gel method utilizing two buffer systems, Kristjansson (1960b) demonstrated the presence of nine serum protein fractions in the medium migration range, the normal area of β-globulin migration. Evidence indicated that two of these fractions were the result of further separation of the previously described B protein. Tests using Nitroso R salt as an iron stain, identified the two fractions as transferrin proteins. Kristjansson (1960b) revised the hypothesis for the genetic control of the observed variation, suggesting a two allele system, each allele controlling the synthesis of one protein fraction. The alleles were postulated to exhibit a dosage effect such that twice as much protein would be synthesized by a double dose of an allele as by a single dose. However, it was felt that these fractions may be comprised of more than two proteins.

Kristjansson (1961) reported a study undertaken to obtain discrete separation of a haptoglobin zone previously observed (Kristjansson 1960b). Vertical starch gel electrophoresis of sera collected from Yorkshire and Landrace swine, revealed the existence of ten haptoglobin fractions three of which exhibited controlled variation. Kristjansson postulated a genetic mechanism controlling the six observed phenotypes involving three allelic genes showing no dominance.

Detailed chemical studies of the genetic differences observed in proteins are invaluable in understanding gene action. In this regard Patras and Stone (1961), using rivanol (2-ethoxy-6,9-diaminoacridine lactate), partially purified bovine serum transferins in an attempt to prepare these proteins in a form which would make chemical study of their electrophoretic differences possible. It was found that treatment of the serum

with rivanol did not differentially affect the starch gel electrophoretic properties of the transferrins.

IV. Genetic Variation in Bovine Transferrins

A. The Genetic Mechanism

Ashton (1957b) discussed the nature of five observed β-globulin types in cattle, and developed a hypothesis for the genetic control of the variation. He postulated that the system was controlled by five pairs of linked genes. Smithies and Hickman (1958), also finding five distinct β-globulin phenotypes, postulated that the variation was best explained on the basis of a single autosomal locus with three alleles. They suggested a genetic explanation for the variation observed, but did consider the possibility of not having found a sixth type.

Later Ashton (1958b) verified the three allele hypothesis proposed by Smithies and Hickman (1958) when he found a sixth β-globulin type. The nature of the phenotypes indicated that no dominance was expressed by any of the three known genes. Thus Ashton (1958b) proposed that the six observed phenotypes represented three homozygous types and three heterozygous types, and that they were under the control of a three allele system with no dominance.

The allelic series controlling the inheritance of the β-globulins was extended by Ashton (1959b) when he observed the presence of two additional alleles in Zebu cattle. All the possible 15 phenotypes were observed except one expected homozygous type. Gene frequencies calculated from matings of Zebu cattle and Zebu X European crosses confirmed the previously unrecognized phenotypes as representing individual genotypes of a five allele system.

Gahne, et al. (1960) and Gahne (1961) described the results of studies of β-globulins in Swedish cattle and reported electrophoretic patterns in agreement with those outlined by Ashton (1958b). In addition Gahne (1961) detected a probable new β-globulin type in two individuals, the migration of two of the slower moving bands being considerably farther than was normally observed.

As previously outlined in this review, the serum β-globulins have been shown to be the specific iron binding proteins of blood serum referred to as transferrins (Giblett, et al. 1959). In the remainder of this dissertation the term transferrin will be employed to refer to the iron binding β-globulins and the same symbol, Tf, as that used to refer to the transferrin locus in humans (Smithies and Hiller, 1959) and in swine (Kristjansson, 1960b) will be applied to the transferrin locus in cattle. The five transferrin alleles hitherto found in cattle can be designated Tf^A, Tf^B, Tf^D, Tf^E and Tf^F (Gahne, 1961), the superscripts being in accordance with those used by Ashton (1958b and 1959b). Following this system of nomenclature, the phenotypes can be designated Tf A-A, Tf A-B, etc., with respective genotypes Tf^A/Tf^A, Tf^A/Tf^B.

B. Gene Frequencies and the Utility of the Transferrin Types

The frequencies of the alleles determining the transferrin types in cattle vary between breeds and between locales. A summary of the frequencies as determined by several workers is presented in Table 1.

Ashton (1958b) noted that the frequency of the Tf^E allele is greater in those breeds originating from the climatically more severe parts of the British Isles. He found the Tf^E allele to be most frequent in cattle of Scottish origin, while it was absent from Jersey and Guernsey cattle, breeds confined mainly to the southern and southwestern areas of

Table 1

A summary of the transferrin gene frequencies found in some breeds of cattle

Breed	Locale	No. of Animals	Gene Frequency Tf^A	Tf^D	Tf^E	Tf^B	Tf^F	
Friesian	Great Britain	214	0.49	0.47	0.04			
Ayrshire	" "	81	0.25	0.64	0.11			
Guernsey	" "	37	0.53	0.47	--			
Jersey	" "	36	0.68	0.32	--			
								Ashton[1]
Shorthorn	" "	108	0.60	0.37	0.03			(1958b)
Hereford	" "	66	0.41	0.57	0.02			
Aberdeen-Angus	" "	38	0.68	0.20	0.12			
Holstein	Ont., Canada	102	0.56	0.37	0.07			Smithies and
Ayrshire	" "	42	0.36	0.36	0.28			Hickman[2] (1958)
SRB[3]	Sweden	1508	0.50	0.21	0.29			
SLB[4]	"	204	0.48	0.50	0.02			Gahne
SKB[5] A)	"	220	0.44	0.54	0.02			(1961)
B)	"	45	0.29	0.54	0.17			
SRB[3]	Sweden	707	0.47	0.23	0.30			Gahne et al. (1960)
Sindhi	Australia	14	0.57	--	0.28	0.04	0.11	
Jersey	"	51	0.51	0.49	--	--	--	
Sindhi X Jersey	"	29	0.52	0.24	0.12	--	0.12	
Sahiwal	"	10	0.10	--	0.15	0.20	0.55	
Sahiwal X Jersey	"	31	0.33	0.28	0.03	0.05	0.31	
								Ashton
Hereford	"	27	0.39	0.52	0.09	--	--	(1959b)
Shorthorn	"	18	0.56	0.39	0.05	--	--	
Brahman X Shorthorn	"	14	0.28	0.43	0.18	--	0.11	
Brahman X Hereford	"	15	0.40	0.24	0.20	--	0.16	
Africander X Shorthorn	"	13	0.19	0.50	0.31	--	--	
Africander X Hereford	"	15	0.23	0.47	0.30	--	--	

3 SRB = Swedish Red and White
4 SLB = Swedish Friesian
5 SKB = Swedish Polled A) Swedish Red Polled
B) Swedish Mountain

1 Data from Milk Marketing Board cattle breeding centres only
2 Recalculated by the writer on the basis of the presently accepted genetic hypothesis

England. Ashton (1958d) also found an association between the frequency of this allele and the location of two Ayrshire herds; in one herd picked at random in the Essex area the frequency of the Tf^E allele was 0.118 $\pm$ 0.025, while in a comparable herd in Aberdeenshire the frequency was 0.261 $\pm$ 0.037. Smithies and Hickman (1958) report a frequency of 0.279 $\pm$ 0.049, in Ayrshires in Ontario, a frequency similar to that found by Ashton in Aberdeenshire. Ashton (1958b) citing these frequencies suggests that transferrin polymorphism in cattle may be concerned with climatic tolerance.

Gahne (1961) working with Swedish cattle was not able to confirm this theory. He found Swedish Polled (SKB), the most northern breed in Sweden and thus located in a colder climate than the other Swedish breeds, to have a lower Tf^E allelic frequency than the Swedish Red-and-White breed (SRB). Gahne notes that the frequency of Tf^E is higher in SRB than in any other European cattle breed so far reported.

Zebu cattle on the other hand, as predicted by Ashton (1958b), also have a high frequency of the Tf^E allele (Ashton, 1959b), an interesting observation in view of the well known climatic and ecological tolerance of these cattle.

The frequency of the transferrin alleles found in Swedish Friesians by Gahne (1961) show no important divergence from those found in Friesians in Great Britain by Ashton (1958b). Smithies and Hickman (1958) report a higher frequency of the Tf^A Allele and a correspondingly lower frequency of the Tf^D allele in Holsteins in Ontario.

Smithies and Hickman (1958) suggest the use of the transferrin types as an economical screening test to precede the expensive and laborious red cell antigen determinations in cases of questionable parentage in cattle. Gahne (1961) and Rendel and Gahne (1961) reported the use of transferrin

types to investigate parentage cases in Swedish cattle and discuss the value of this polymorphism in supplementing red cell antigen tests.

The analysis of serum transferrins is of value in the diagnosis of zygosity in cattle twins. In twin diagnosis, the red cell antigen test is often difficult to interpret because of erythrocyte mosaicism whereas the transferrin type is not influenced by the fetal vascular anastomosis (Gahne, et al. 1960; Gahne, 1961). Since dizygous twin pairs may differ in their transferrin type, this analysis provides a short-cut method by which at least a part of the dizygous pairs may be detected.

C. Transferrin Types and Their Relation to Production Traits

Ashton (1959a) reported that the transferrin genotype of the parents influenced the proportion of the two genotypes expected from some reciprocal matings. The analysis of matings involving Tf^A and Tf^D alleles in both Ayrshire and Holstein cattle revealed an excess of offspring "like the mother" in transferrin type. In addition, similar matings involving the Tf^E allele demonstrated that when this allele was present in the sire's genotype only, an excess of offspring "like mother" occurred, and that when the Tf^E allele was present in the dam's genotype only, a consistent lack of Tf^E carrying offspring occurred. Ashton postulated that these asymmetrical segregation ratios were the result of embryonic mortality, and that incompatability between mother and fetus of different transferrin genotypes was the cause of the embryonic mortality. Gahne (1961) reporting the results of 140 reciprocal matings in which one of the parents was homozygous found only a very slight deviation from the 1:1 ratio expected in the offspring.

Later work (Ashton 1961) using data collected from an artificial insemination program involving Australian Illawarra Shorthorn and Australian Jersey cattle showed that matings between unlike homozygotes, matings which

would result in embryos "unlike-mother", are as fertile as matings between like homozygotes, matings which would result in embryos "like-mother". These findings rendered the original hypothesis for the occurrence of asymmetrical segregation ratios untenable. Ashton (1961) rationalized the hypothesis by postulating that heterozygotes per se are at an advantage 'in utero' and that this would compensate for their being "unlike-mother". The compensatory effect would have to be of the same order of magnitude as the "like-mother" effect. Ashton (1961) was able to show, however, that matings between homozygotes were more fertile than matings involving one or two heterozygotes, indicating that transferrin type does influence breeding efficiency, the latter being defined as the percentage of individual inseminations resulting in a live or stillborn calf or an aborted fetus.

The findings reported by Ashton (1961) represented an unusual genetic situation because it would appear that heterozygotes are at a disadvantage with regard to fertility. He concluded, however, that since the number of heterozygotes found in unrelated populations has in several instances been greater than that expected from gene frequency calculations, it seems probable that heterozygotes are more viable, and that this superiority would be sufficient to produce a stable polymorphism.

In another study, Ashton (1960b) concluded that the transferrin locus is concerned in the genetic control of milk yield. The mean genetic value of the Tf^D allele over the Tf^A allele was estimated at about one sixth of the total genetic variation in milk yield for the major milk breeds of England and Wales. The transferrin phenotypes and their relation to the distribution of butterfat percentage was also investigated. No difference was found between the mean first lactation butterfat percentage of cows with phenotypes Tf A-A, Tf A-D, and Tf D-D, although there was some indication that the mean percentage of Tf A-E and Tf D-E cows was higher than the

mean of the other groups.

Any postnatal selection technique which increases the chances of rearing animals of higher than average production is potentially valuable; but, as suggested by Ashton (1960b), it is not possible to predict from transferrin type the ability of an individual bull to produce better than average milk yielding daughters because the inherited level of production in an individual heifer is governed by many sets of genes of which the transferrin alleles are presumably one.

EXPERIMENTAL

I. Objectives

The University of Alberta established the foundation for an extensive beef breeding research project during the summer and fall of 1960. The project is located on approximately 5600 acres of land at Kinsella, 95 miles east of Edmonton, Alberta. The females for the project are divided into two herds, one a Hereford line, the other a hybrid line involving a combination of Angus, Galloway and Charolais breeds.

The aims of the project have been outlined in general terms by Berg (1962):

"The main objective of the program is to study, in detail, inheritance in cattle in order to gain a better understanding of how to improve their efficiency and productivity.

"A second goal is to evolve improved and practical methods of breeding and selection which can be adopted by cattle producers, enabling them to more fully apply science to their breeding operations.

"An attempt will also be made to cast some light on the value of hybridization in the foundation for improved strains of beef cattle.

"Some of the cattle will be available for detailed studies in physiology, genetics and nutrition in the Department of Animal Science laboratories at the University."

In keeping with the Beef Breeding Research Project, the studies reported herein were undertaken to:

1. Determine the genetic structure of the cattle as regards the transferrin locus.
2. Determine the relation of the transferrin genotypes to

efficiency and productivity.

II. Sources and Description of Data

A. The Hereford Herd

The Hereford line is comprised of foundation females obtained from four breeders and from the University Research Farm in Edmonton. The breeders who supplied foundation females were McIntyre Ranching Company, Magrath; Norman Babey and Son, Lloydminster; B. and R. Powlesland, Del Bonita; and H. Porteous, Madden.

Thirty-one Hereford calves were born during the spring of 1961, twenty-eight of which were available for transferrin typing in the spring of 1962. During the spring of 1962 fifty-four Hereford calves were born, fifty-two of which were available for transferrin typing in the same year.

B. The Hybrid Herd

The hybrid herd is comprised of foundation females of three breeds obtained from five breeders and from the University Research Farm in Edmonton. The five breeders and the breed supplied by each is tabulated below.

Breeder	Breed
I. Ohler, Carmangay	Charolais X Angus
J. Bartsch, Milo	Charolais X Angus (calves)
E. Blades, Nanton	Galloway
A-7 Ranch, Nanton	Galloway
Glenbow Ranches, Cochrane	Angus
University Research Farm	Angus

Forty calves were born in the hybrid herd during the spring of 1961. Thirty-one of these, plus twelve female calves obtained from J. Bartsch were available for transferrin typing during the spring of 1962. Sixty-three were born in 1962, sixty-two of which were available for transferrin typing in the same year.

C. General Management of the Herd

The project is operated as a practical ranch operation within the limits of research technique. The cows are bred in July and August, and the calves are weaned in October. Creep feeding is not practiced.

The cows are managed as a single herd, except during part of the breeding season. The cows are encouraged to graze as much as possible during the winter, with minimal feeding of roughage being practiced. In addition a limited amount of a pelleted ration is fed on the range as an energy, protein, and vitamin A supplement.

The calves are wintered on a growing ration, with access to native pasture and trees. No artificial shelter is supplied other than straw bedding.

Both natural and artificial breeding were practiced in 1960 and 1961, the cows being hand bred during the first two-thirds of the breeding season, after which time the bulls were placed with the cows.

III. Blood Serum Typing

An outline of the methods employed in the collection and processing of the blood, starch gel electrophoresis of the serum, and the photographing of the starch gels will be found in Appendix A. Blood samples were collected at the University Ranch at Kinsella and transported to the Animal Science laboratories at the University of Alberta where the procedures relating to the processing of the blood were carried out. The starch gel electrophoretic procedures were also carried out at the Animal Science laboratories as were the procedures involved in photographing the starch gels.

IV. Preliminary Experiments

A preliminary experiment was undertaken during the initial stages of the work to familiarize the writer with the starch gel electrophoretic

method and to establish a standard technique which could be used in subsequent analyses. The method of Smithies (1959a) was used initially, employing human serum obtained from the Provincial Laboratory of Public Health, University of Alberta. Repeatable results similar to those reported by Smithies (1959a) were achieved.

An attempt was then made to establish conditions of starch gel preparation which would give the most desirable separation of bovine serum. Serum was obtained from three pairs of identical twins located at the University Research Farm.

A factorial experiment was designed involving four starch gel concentrations ranging from 12% to 14% and five buffer pH levels ranging from 8.55 to 8.75. Two starch gels of each type were prepared, using the method of Smithies (1959a). A boric acid-sodium hydroxide buffer was used and the ionic strength maintained at approximately 0.1 for all pH levels.

The results of the factorial experiment suggested that optimum separation could be achieved using a starch concentration of 13% and a buffer pH of 8.65. The composition of this buffer was 0.026 M boric acid and 0.01 M sodium hydroxide. The bridge solution selected was that used by Smithies (1959a) containing 0.3 M boric acid and 0.06 M sodium hydroxide at a pH of 8.45. It was found that this buffer used as the bridge solution resulted in a minimum of shrinkage at the anodic end of the gel.

The analysis of the bovine serum was repeatable from one run to the next, and the transferrin pattern of the members of twin pairs was identical in all cases. One twin pair suspected of not being monozygotic exhibited slight differences in the intensity of the transferrin bands and of the $F\alpha_2$ globulin band although the transferrin patterns were identical. This difference in stain intensity suggested that the pair may not in fact be

monozygotic. It was later shown by the supplemental use of other criteria that the pair was probably dizygotic (Mendel, 1962).

The procedure outlined by Kristjansson (1961) was employed to determine the possibility of locating a haptoglobin fraction in bovine serum. Initially, serum samples were obtained from 12 hybrid pigs in the swine herd maintained at the University Research Farm. Results similar to those reported by Kristjansson (1961) were obtained.

When the procedure was applied to the analysis of bovine serum proteins it was found that hemoglobin and/or a haptoglobin fraction migrated in the same area as the transferrin fraction. Attempts at improving the separation by varying the pH of the tris (hydroxymethyl) aminomethane-citric acid buffer used by Kristjansson were not successful. Further work is required before a definite conclusion can be reached as to the presence of a haptoglobin fraction in bovine serum.

V. Experiment 1: Gene Frequency Studies of the Transferrin Locus in the University Ranch herds

A. Object

To determine the transferrin locus gene frequencies in the University Ranch cattle herds and to study the significance of any polymorphisms exhibited.

B. Methods and Materials

The calves born in the spring of 1961 in both the Hereford and hybrid herds were available for blood sampling in April of 1962. Blood samples were obtained from 28 calves in the Hereford herd and 53 calves in the hybrid herd. The samples were processed as outlined in Appendix A and the sera stored in sealed vials at -5^{o} C.

Calves born in the spring of 1962 were not sampled until July to allow the animals to mature as much as possible. Samples were obtained from 52 calves in the Hereford herd and from 62 calves in the hybrid herd.

The available bulls, 2 in the Hereford herd and 3 in the hybrid herd, as well as 75 cows in the Hereford herd and 76 cows in the hybrid herd were sampled in June of 1962. The samples taken in June and July were also stored at $-5^{o}C$.

The electrophoretic analysis of the serum samples was carried out during the summer of 1962, and the results recorded by photographing the gels. Sets of four starch gel trays were run twice daily, one set from 8 a.m. to 8 p.m. and the other from 8 p.m. to 8 a.m. allowing for the possible analysis of 48 samples per day. All the samples were analysed at least twice. A standard serum was not used unless sufficient diversity of transferrin types failed to occur at random in any one tray to allow for convenient serum typing. If typing could not be suitably carried out, a third run was made in which a standard Tf A-E, was included.

When the electrophoretic analysis was completed, the photographs (Fig. 3) which had been obtained in duplicate were labelled by sample number. One photograph of each starch gel was cut into strips so that each strip consisted of the photograph of a single sample. Transferrin typing then proceeded using the remaining photograph. The strips representing single samples were grouped according to their transferrin type. Once all the samples had been typed, the procedure was repeated using the photographs of the duplicate electrophoretic analysis. Any discrepancies in typing which occurred were further assessed using the photographs from both analyses and a final typing was made.

After the transferrin type of each sample had been established, the strips representing single samples from one electrophoretic run were

grouped according to sire. Thus the cows and offspring were arranged in mating classes consisting of sire, dam and calf.

Since not all of the bulls used in 1961 and none of those used in 1960 were available for blood sampling, an attempt was made to derive the genotype of the bulls from the genotypic pairing of dams and calves. Using a procedure of allele elimination, it was possible to establish the genotypes of all the bulls used in 1961, but the number of offspring available was not sufficient to determine the genotypes of those used in 1960 (sires of the 1961 calf crop). The typing determined electrophoretically for the five available bulls (sires of 1962 calves) was used to test the accuracy of the derivation of their genotypes. No discrepancies were noted.

Gene frequencies were calculated for each breed and each herd from the relationships, $p = (2\ Tf\ A\text{-}A + Tf\ A\text{-}D + Tf\ A\text{-}E)/2N$, $q = (2\ Tf\ D\text{-}D + Tf\ A\text{-}D + Tf\ D\text{-}E)/2N$, and $r = (2\ Tf\ E\text{-}E + Tf\ D\text{-}E + Tf\ A\text{-}E)/2N$, where Tf A-A, Tf A-D, etc. are the numbers of animals of phenytype Tf A-A, Tf A-D, etc., found in any one group and N is the total number of animals in the group, and where p, q and r represent the frequencies of genes Tf^A, Tf^D, and Tf^E respectively.

The electrophoretic pattern of each sample from the point of origin to the tailing edge of the albumin was examined critically, using the description of Smithies and Hickman (1958) as a guide, and any apparent deviations were noted.

C. Results and Discussion

1. Characteristics of the transferrin types

Six distinct transferrin phenotypes were observed after starch gel electrophoresis. The types are illustrated schematically in Figure 2 and

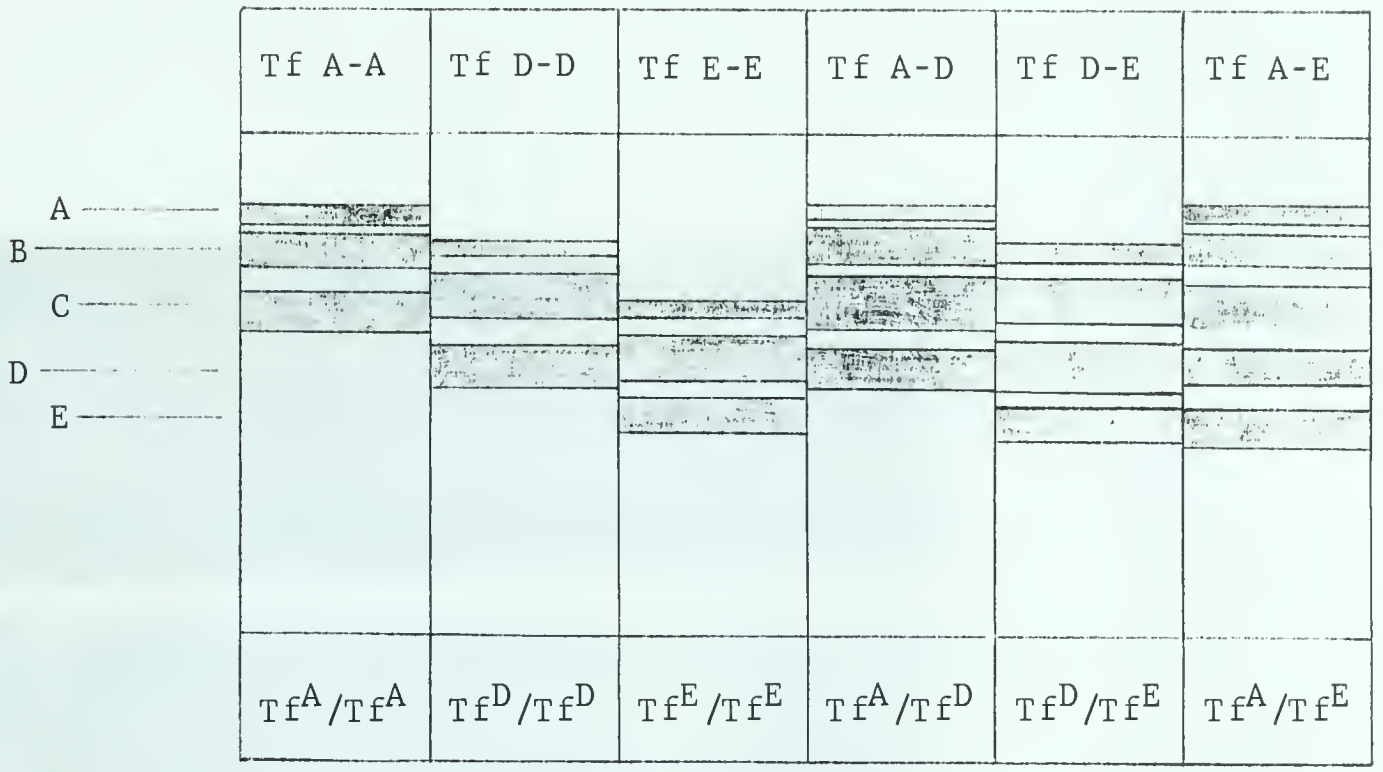

Fig. 2. A schematic representation of the six transferrin types. The genotypes are shown below and the phenotypes above the corresponding transferrin pattern. The transferrin bands are labelled A, B, C, D and E, in order of decreasing mobilities.

are shown in a photograph of representative samples of the six types in a single starch gel in Figure 3. In the figures, the proteins labelled A, B, C, D, and E, vary in occurrence and amount from type to type. Five of the types have been described in detail by Smithies and Hickman (1958). The characteristics of the bands in the sixth type, Tf E-E, are very similar to those in types Tf A-A and Tf D-D. One distinctive character of type Tf E-E besides migration rate is the relatively large quantity of protein present in the intermediately migrating band as compared to the corresponding band in types Tf A-A and Tf D-D.

The bands in types Tf A-D and Tf D-E are also very similar except in their relative migration rates. As in type E-E, the intermediately migrating band (band D) in type Tf D-E, contains a proportionally greater

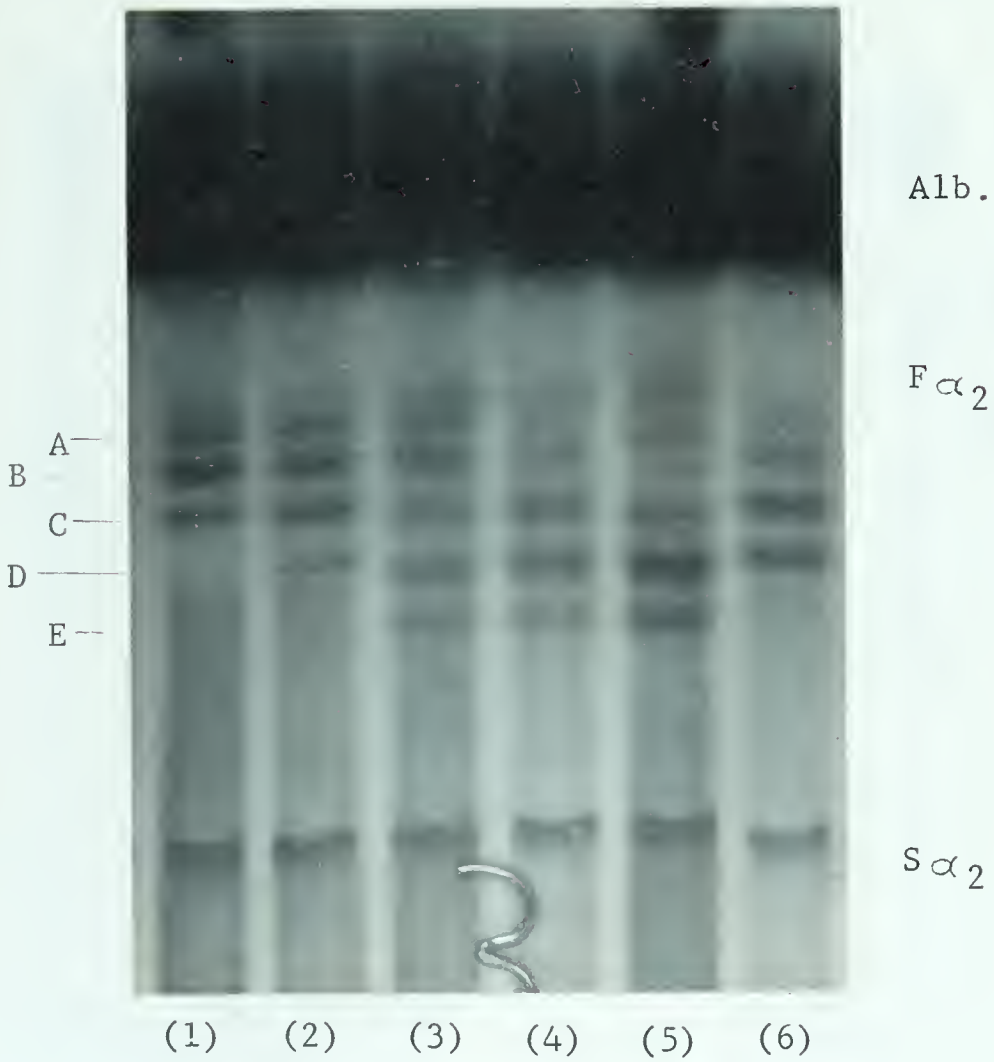

Fig. 3. Photograph of results of starch gel electrophoresis of representative samples of each of the six transferrin types compared in a single starch gel. The five transferrin bands are labelled A, B, C, D and E. 1. Tf A-A, 2. Tf A-D, 3. Tf A-E, 4. Tf D-E, 5. Tf E-E, 6. Tf D-E.

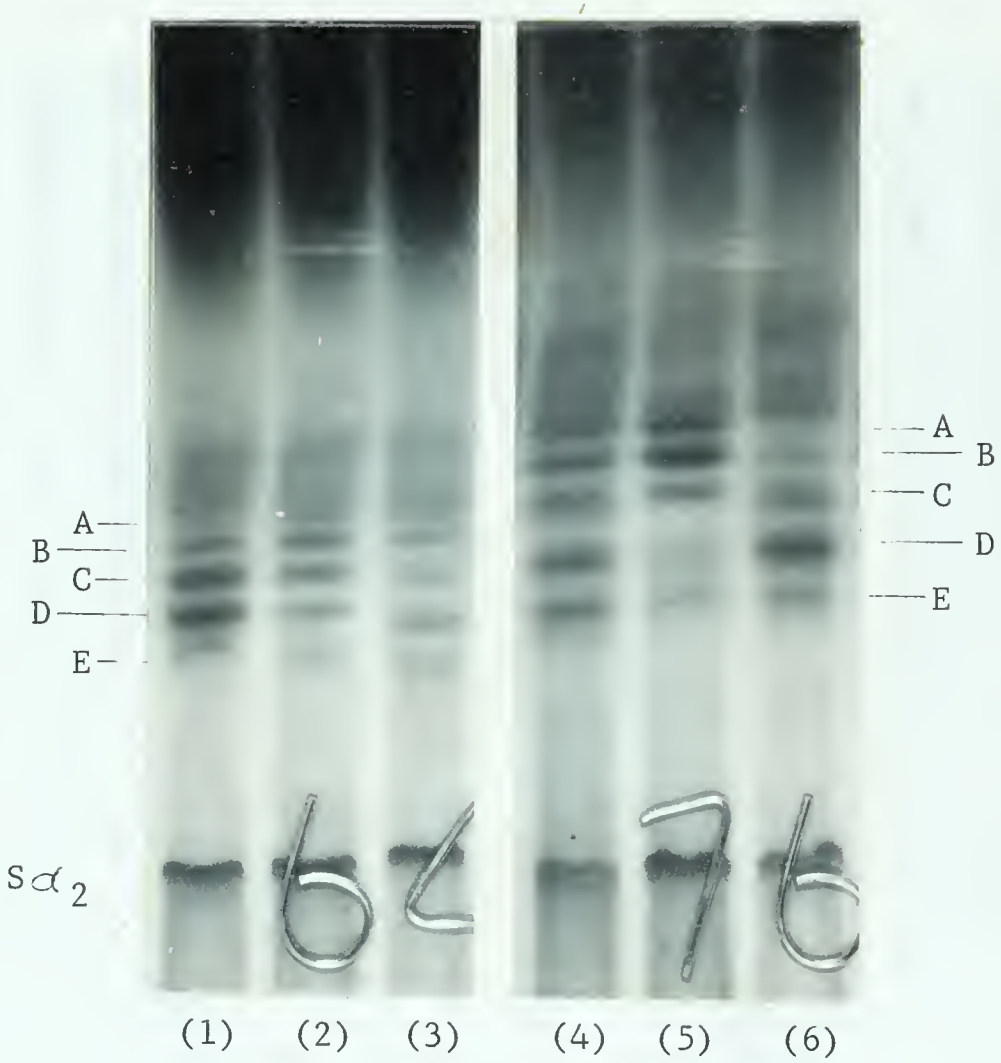

Fig. 4. Photograph of a portion of two starch gels illustrating some of the variation observed in transferrin types. 1. Tf D-D, 2. Tf A-D, 3. Tf A-E, 4. Tf A-E, 5. Tf A-A, 6. Tf D-E. Alb. = Albumin, $F\alpha_2$ = Fast α_2-globulin, $S\alpha_2$ = Slow α_2-globulin.

quantity of protein than the corresponding band in type Tf A-D. All five bands are present in type Tf A-E. However, the quantity of protein in each band appears to be somewhat less than that present in the corresponding bands of the other types. The centrally migrating band (band C) tends to separate into two fractions during extended electrophoresis.

2. Genetic control of the transferrins

Smithies and Hickman (1958) and Ashton (1958b) suggested that the simplest genetic mechanism for the control of the observed variation in the transferrins requires a three allele system with no dominance. The action of the alleles must then be assumed to be as follows using the symbols Tf^A, Tf^D and Tf^E to designate the alleles. Allele Tf^A leads to the presence of transferrins A, B, and C, in the proportions illustrated by serum type Tf A-A; allele Tf^D to transferrins B, C, and D, in the proportions illustrated by serum type Tf D-D; and allele Tf^E to transferrins C, D, and E, as in serum type Tf E-E.

Thus, types Tf A-A, D-D and E-E are recognizable as genotypes Tf^A/Tf^A, Tf^D/Tf^D, and Tf^E/Tf^E, respectively, and types Tf A-D, D-E and A-E as composites of genotypes Tf^A/Tf^D, Tf^D/Tf^E, and Tf^A/Tf^E, respectively. The quantity of transferrin present in each of types Tf A-A, D-D, and E-E being proportionally equal suggests that the variation in gene action exhibited by the three alleles is the result of differences in protein type not of differences in protein quantity.

The main duplicated bands, transferrin C in type Tf A-D and transferrin D in type Tf D-E, contain a greater quantity of protein than the other main components of these types. The protein in band C of type Tf A-D appears to migrate as a unit while the protein in band D of type Tf D-E migrates as a broadened band, less clearly defined at the tailing edge.

Band C of type Tf A-E, as was previously noted, tends to separate into two components. The nature of the bands, C in types Tf A-D and A-E and D in type D-E, suggests that the alleles act in an additive manner and that the proteins resulting from the presence of Tf^E are not identical to the corresponding proteins resulting from the presence of Tf^A or Tf^D. It has been observed when comparing types Tf D-D, D-E, and E-E on a single starch gel that the three bands of type E-E migrate somewhat more slowly than those of type D-D. It has also been observed that mixtures of sera of two homozygous types result in a migration pattern like that of the corresponding heterozygous type except that the amount of protein present appears to be greater.

Transferrin patterns have been observed which do not conform completely to the above generalized patterns. Examples of these are shown in Figures 3 and 4. The transferrin pattern shown in Fig. 4 (5) has been typed as Tf A-A on the basis of the characteristics of bands A, B, and C. However, bands D and E are also present though in very small amounts. Similarly, the pattern shown in Fig 3 (5) has been typed as Tf E-E on the basis of bands C, D, and E, but it is clear that bands A and B are also present. Similarly, patterns typed as Tf A-D have been observed with the additional band E, Fig 4 (2), and patterns typed as Tf D-E with the additional band A. The density of staining of the additional bands is often very near the limits of perceptibility, but it does not seem likely that they are artifacts.

It is very difficult to explain the presence of these additional bands on the basis of the gene actions described above. It would appear that at least part of the time all five transferrins are present and that the particular gene present regulates the relative amounts of the trans-

ferrins synthesised. The synthesis of these proteins must be assumed to be far removed from what might be considered direct gene control. Secondly, it is probable that more than one common anabolic reaction is involved in the synthesis of these electrophoretically different proteins, at least in the latter stages. Transferrin synthesis, therefore, must be influenced by a relatively large segment, or segments, of genetic material. With the information presently available, it would be a fallacy to restrict the variation possible in such a system to a simple one gene - three protein relation with no intermediate types. Four main questions must be answered: (1) At what step in transferrin metabolism does the Tf locus influence transferrin synthesis? (2) What is the nature of the action of the Tf locus? (3) What alteration in this action results from a mutation at the Tf locus? and (4) What influence does the physiological state of the animal have on the amount of protein present in the blood? Obtaining information on the amount of the various types of protein present in the serum of different animals is important from the standpoint of setting limitations on the accuracy of protein detection. The quantity of the protein types found in trace amounts, and thus the ease of detecting these types may be dependent upon the total concentration of protein in the serum. The concentration of protein in serum can be expected to vary from animal to animal regardless of transferrin type.

A second unusual pattern was observed which exhibited an arch shaped band and migrated in the area of transferrin E. An example is shown in Fig. 4 (1). It was possible to group the patterns exhibiting this band into two types, one showing a band dark in staining intensity as illustrated in Fig. 4 (1) and one showing a band intermediate in staining intensity. Eleven such patterns were observed in the Hereford herd, six intensely staining and five intermediately staining. Fifteen were observed in the

hybrid herd, six intensely staining and nine intermediately staining. It is interesting to note that of those observed in the hybrid herd all six intensely staining patterns were observed in samples from Angus cows. Two of the intermediately staining patterns were also observed in samples from Angus cows and the remaining seven in samples from Galloway cows. A similar pattern was observed in samples from nine calves of the 1962 hybrid herd and in the sample from one bull, an Angus. It was not determined whether or not this protein was a member of the β-globulin complex.

3. Gene frequency studies

The frequencies of the genes Tf^A, Tf^D and Tf^E were calculated from the transferrin typing data using the three allele hypothesis established by Ashton (1958b). The frequencies found in the Hereford and hybrid herds are summarized in Table 2, and those found in the offspring born in 1961 and 1962 in Tables 3 and 4, respectively.

Great variation exists within the hybrid herd and between the hybrid and Hereford herds (Table 2). Noteworthy is the complete absence of the Tf^E allele from the Hereford herd. Ashton (1958b) reported a very low frequency of this gene in British Hereford cattle (See Table 1 of Literature Review). Although the Hereford herd is comprised of cattle obtained from five different sources throughout the province, it is possible that the Tf^E allele does exist in Alberta Herefords but at a very low frequency.

The Tf^E allele was not found in either the 1961 or 1962 Hereford offspring indicating that probably none of the five sires of the 1961 calves or the three sires of the 1962 calves carried the Tf^E allele. This was found to be true for the sires of the 1962 calves all three of which

Table 2

Frequencies of genes Tf^A, Tf^D and Tf^E found in the Hereford and hybrid herds, for the year 1962

	No.	Tf^A	Tf^D	Tf^E
Cows - Hybrid Herd				
Galloways - A-7	26	0.269	0.500	0.231
Galloways - Blades	9	0.500	0.500	0.0
Combined	35	0.328	0.500	0.171
Angus - U.A.	12	0.583	0.417	0.0
Angus - Glenbow	19	0.395	0.500	0.105
Combined	31	0.468	0.468	0.064
Charolais X Angus - Ohler	10	0.300	0.650	0.050
Total Cows	76	0.382	0.506	0.112
- - - - - - - - -				
Bulls - Hybrid Herd				
Angus	2	0.250	0.500	0.250
Galloway	3	0.500	0.167	0.333
Charolais	2	0.0	0.500	0.500
Total Bulls	7	0.286	0.357	0.357
- - - - - - - - -				
Cows - Hereford Herd				
U.A.	21	0.524	0.476	0.0
McIntyre	23	0.435	0.565	0.0
Babey	10	0.550	0.450	0.0
Powlesland	8	0.500	0.500	0.0
Porteous	13	0.346	0.654	0.0
Total Cows	75	0.467	0.533	0.0
- - - - - - - - -				
Bulls - Hereford Herd	3	0.500	0.500	0.0

Table 3

Frequencies of genes Tf^A, Tf^D and Tf^E
found in calves born in 1961

Breed[1]	No.	Tf^A	Tf^D	Tf^E
Galloway	26	0.404	0.288	0.308
Charolais X (Charolais X Angus)	8	0.500	0.438	0.062
Charolais X Angus	16	0.531	0.438	0.031
Angus	3	0.667	0.333	0.0
Total Hybrid Calves	53	0.472	0.358	0.170
Total Hereford Calves	28	0.428	0.571	0.0

1 Breed of sire listed first

Table 4

Frequencies of genes Tf^A, Tf^D and Tf^E
found in calves born in 1962

Breed[1]	No.	Tf^A	Tf^D	Tf^E
Angus X Galloway	22	0.250	0.614	0.136
Galloway X Angus	13	0.423	0.308	0.269
Charolais X Galloway	6	0.083	0.833	0.083
Charolais X Angus	14	0.286	0.607	0.107
Galloway X (Charolais X Angus)	7	0.500	0.357	0.143
Total Hybrid Calves	62	0.500	0.357	0.143
Total Hereford Calves	52	0.538	0.462	0.0

1 Breed of sire listed first

were established to have Tf A-D phenotypes. The frequency of the two alleles present in the Hereford herd, Tf^A and Tf^D, was found to be very near a 50:50 distribution.

The allelic frequencies in the hybrid herd are disproportionate (Table 2). Although the numbers are limited it is interesting to note the absence of the Tf^E allele from the Blades Galloway and U.A. Angus cattle. These two groups exhibit a near 50:50 distribution of the Tf^A and Tf^D alleles, while groups in which the Tf^E is present, exhibit an excess of the Tf^D allele. The frequency pattern for the overall hybrid herd shows a distinct lack of the Tf^E allele and a definite excess of the Tf^D allele.

The gene frequency pattern of the 1962 hybrid calves is not appreciably different from that of the hybrid herd since the sires carried all three alleles in nearly equal frequency. The higher frequency of the Tf^A allele apparent in the 1961 calves may possibly be explained on the basis of chance, considering the small numbers involved. At least two of the five bulls used to sire these calves were heterozygous for Tf^A, and one other heterozygous for Tf^E. The dams of the Bartsch Charolais X Angus calves were not available for transferrin typing, so the gene frequency pattern of the dams in the hybrid herd is not complete for comparison with the frequencies of the 1961 calves.

Complete parentage data were available for 50 Hereford and 59 hybrid calves born in 1962. The distribution of the progeny genotypes from the available mating classes is summarized in Tables 5 and 6. There is no discrepancy between the observed and expected distributions of the three possible genotypes in the Hereford herd ($P > 0.95$). The difference between the observed and expected distributions of the progeny genotypes

of the 1962 calves in the hybrid herd is not significant (P 0.20). However it should be noted that the Tf^E allele appears at a lower level than expected, whereas the Tf^D allele appears at a higher level, particularly in the Tf^A/Tf^D heterozygote. The high frequency of occurrence of the Tf^A/Tf^D heterozygote may be indicative of how the heterozygotes are maintained in the population. Ashton (1961) reported that the number of heterozygotes in random samples from unrelated cow populations has been found in several instances to be greater than expected from gene frequency calculations. A similar effect was noted by Gahne et al. (1960) but Gahne (1961) was unable to confirm these findings.

Table 5

Distribution of 1962 Hereford progeny genotypes (observed/expected) from various mating classes and the results of X^2 test of their fit to the three allele hypothesis

Mating[1]	Number of Matings	Genotypes of offspring Tf^A/Tf^A	Tf^D/Tf^D	Tf^A/Tf^D	P of X^2
[2]A-A X A-D	11	7/5.5		4/5.5	
D-D X A-D	9		5/4.5	4/4.5	
A-D X A-D	30	7/7.5	7/7.5	16/15.0	
Total	50	14/13.0	12/12.0	24/25.0	0.95

1 Only those matings observed are included
2 Symbol Tf assumed in phenotypic designations

Ashton (1959a, and 1961) suggested that embryonic mortality in the bovine is influenced by the transferrin type of the sire and dam. The distribution of the progeny transferrin genotypes found in the Hereford and hybrid herds are shown in Table 7 and Table 8, respectively.

Table 6

Distribution of 1962 hybrid progeny genotypes (observed/expected) from various mating classes and the results of X^2 test of their fit to the three allele hypothesis

Mating	Number of Matings	Genotype of Offspring					
		Tf^A/Tf^A	Tf^D/Tf^D	Tf^E/Tf^E	Tf^A/Tf^D	Tf^D/Tf^E	Tf^A/Tf^E
[2]A-A X D-D	2				2/2		
A-A X D-E	3				3/1.5		0/1.5
A-A X A-E	2	1/1					1/1
D-D X D-D	1		1/1				
D-D X E-E	1					1/1	
D-D X A-D	6		3/3		3/3		
D-D X D-E	9		6/4.5			3/4.5	
D-D X A-E	8				5/4	3/4	
A-D X A-D	2	0/0.5	0/0.5		2/1		
A-D X D-E	9		3/2.25		5/2.25	0/2.25	1/2.25
A-D X A-E	10	2/2.5			3/2.5	2/2.5	3/2.5
D-E X D-E	1		0/0.25	0/0.25		1/0.5	
D-E X A-E	4			0/1	2/1	1/1	1/1
A-E X A-E	1	0/0.25		0/0.25			1/0.5
Total*	59	3/4.25	13/11.50	0/1.50	25/17.25	11/15.75	7/8.75

* P of X^2, $0.25 > P > 0.20$

1 Only those matings observed are included

2 Symbol Tf assumed in phenotypic designation

Table 7

Distribution of "Like-Mother" genotype (observed/expected) in 1962 Hereford progeny and the results of X^2 test of their fit to the expected ratio

Mating		Number of Matings	Number "Like-Mother"	Number "Unlike-Mother"	P of X^2
Sire	Dam				
Tf A-D	Tf A-A	11	7/5.5	4/5.5	
Tf A-D	Tf D-D	9	5/4.5	4/4.5	
Tf A-D	Tf A-D	30	15/15	15/15	
Total		50	27/25	23/25	>0.50

Table 8

Distribution of "Like-Mother" genotype (observed/expected) in 1962 hybrid progeny of various mating classes and the results of X^2 test of their fit to the expected ratio

Sire	Dam	Number of Matings	Number "Like-Mother"	Number "Unlike-Mother"	P of X^2
Tf D-D	Tf A-D	6	3/3	3/3	
Tf D-D	Tf D-E	4	1/2	3/2	
Tf D-E	Tf D-D	5	3/2.5	2/2.5	
Tf A-E	Tf A-A	2	1/1	1/1	
Tf A-D	Tf A-D	2	2/1	0/1	
Total[1]		19	10/9.5	9/9.5	>0.80
Tf D-E	Tf A-D	9	4/2.25	5/6.75	
Tf A-E	Tf A-D	7	2/1.75	5/5.25	
Tf A-E	Tf D-E	3	1/0.75	2/2.25	
Total[2]		19	7/4.75	12/14.25	>0.20
All Matings		38	17/14.25	21/23.75	>0.30

1 Matings involving 1:1 expected ratio
2 Matings involving 1:3 expected ratio

The distribution observed in the Hereford herd does not differ significantly from the expected. The distribution in the hybrid herd does not differ from the expected in matings which result in an expected 1:1 ratio of genotypes like to unlike dam. Although the differences are not statistically significant there is an indication of an excess of offspring with genotypes like the dam in matings which would be expected to yield a 1:3 ratio of like to unlike dam. These results tend to agree with those reported by Ashton (1959a), as he found an excess of offspring with genotypes like that of the dam.

Table 9

Occurrence of Tf^E allele in progeny (observed/expected) of some matings in 1962 hybrid herd when the Tf^E allele is present (1) in the sire genotype only, (2) in the dam genotype only, and the results of X^2 test of the fit to the expected 1:1 ratio

Sire	Dam	Number of Matings	Progeny Genotypes With Tf^E	Progeny Genotypes Without Tf^E	P of X^2
1. Tf^E in sire only					
Tf A-E	Tf A-D	7	3/3.5	4/3.5	
"	Tf A-A	2	1/1	1/1	
"	Tf D-D	7	4/3.5	3/3.5	
Tf D-E	Tf A-A	2	0/1	2/1	
"	Tf D-D	5	2/2.5	3/2.5	
"	Tf A-D	9	1/4.5	8/4.5	
Total		32	11/16.0	21/16.0	>0.10
2. Tf^E in dam only					
Tf D-D	Tf D-E	4	1/2	3/2	
"	Tf A-E	2	0/1	2/1	
Total		6	1/3	5/3	>0.20

The frequency of occurrence of the Tf^E allele in the progeny genotypes of matings in the hybrid herd is summarized in Table 9. It

is evident that the occurrence of the Tf^E allele was at a disadvantage in the herd examined at least when the Tf^E allele was present in the sire's genotype only. The same trend was apparent when the Tf^E allele was present only in the dam's genotype, but the numbers were not sufficient to exclude chance segregation. Ashton (1959a) found that an excess of offspring of genotype like the mother occurred when the Tf^E allele was present in the sire's genotype only, and an excess of offspring with genotypes without the Tf^E allele when the Tf^E allele was present only in the dam's genotype. The results of this study tend to support Ashton's findings.

D. Summary

1. Using starch gel electrophoresis the transferrins in two cattle herds were studied. Three phenotypes were observed in a Hereford herd and six in a hybrid herd comprised of Galloway, Angus and Charolais breeds.

2. The characteristics of the transferrin types and the postulated three allele genetic mechanism for the control of the observed variation are discussed.

3. Gene frequencies were calculated for each herd. Great variation was found in the frequencies of the transferrin alleles within the hybrid herd and between the hybrid and Hereford herds. The Tf^E allele was not found in the sera of the Hereford cattle. The frequencies of the transferrin alleles found in the cows in the Hereford herd were $Tf^A = 0.467$ and $Tf^D = 0.533$ and in the cows in the hybrid herd were $Tf^A = 0.382$, $Tf^D = 0.506$ and $Tf^E = 0.112$.

4. Parentage data were in general agreement with the theory of inheritance, although the data indicated that offspring in the hybrid herd carrying the Tf^E allele occurred at a lower level than expected.

5. The possible relation of transferrin type to embryonic mortality

was studied. The results were in general agreement with those of other workers, indicating a possible interaction between the genotypes of the fetus and dam.

VI. Experiment II: Statistical Analysis of Production Traits with Special Reference to Transferrin Type

A. Object

To determine the effect of transferrin genotype on some production traits of beef cattle and to determine, concurrently, the effect of sire, sex of calf, and breed, source and age of dam on these traits.

B. Source and Description of Data

The University of Alberta beef breeding project includes a performance testing program which attempts to evaluate and correlate the performance of each animal during five stages: (1) preweaning; (2) post-weaning in the first winter, on a growing type ration; (3) summer grazing; (4) second winter, hardiness on a low maintenance ration; and (5) lifetime reproduction and production. The data collected in the initial stages of the program, namely from the spring of 1961 through October of 1962, were available for this study.

The first calf crop was born in the spring of 1961. Calves were routinely weighed at birth. To obtain preweaning performance data the calves were weighed twice during the summer, and again at weaning in October. Weaning weight adjusted to 180-days was calculated from these data (calculated 180-day weight).

In the winter of 1961-62, the available calves were carried on a growing test to obtain postweaning performance data. The calves were divided by sex and fed a growing ration consisting of oats (13.2% crude protein) and mature grass hay (6.5% crude protein). Beginning about the end of January, 3 lb. of pelleted ration (18.8% crude protein) was fed per calf per week. The initial weight of the calves was taken on November 7th. They were subsequently weighed every 28 days and a final weight, the average of three daily weighings, taken at the end of a 154 day period.

Yearling weight adjusted to 365 days was calculated from these data (calculated 365-day weight).

The calves born in 1962 were similarly treated. They were weighed at birth, twice during July, once in September, and at weaning in October. Weaning weight adjusted to 180-days was calculated from these data.

The cows in the two herds maintained at the University Ranch were weighed at five stages during the year; February - a mid-winter weight, April - a spring, precalving weight, June - a post-calving weight, August - a breeding, preweaning weight, and October - a post-weaning weight. An estimation of a cow's performance as to weight loss and/or gain during the wintering and nursing periods was derived from these data. In this study the estimates are based on the accumulated weight loss over the three periods from August, 1961 through February and April to June, 1962. A standard cow weight for the period was also calculated as an average of the October and June weights.

A number of yearling and two year old heifers were dehorned on October 3rd, 1961, the remaining animals being either polled or previously dehorned. Since gestation may influence an individual's performance during the wintering period, those animals not calving in 1962 were recorded separately.

The transferrin type of each individual was obtained as described in Experiment I. Only those animals with clearly established parentage and transferrin type and with complete performance records were included.

C. Method of Analysis

The data collected in this study were non-orthogonal. Thus a special treatment was employed to permit inferences.

Unbiased estimates of the effects of such factors as transferrin

genotype, breed, and sex on production traits were obtained by the method of least squares in the manner described in Appendix B. The least squares estimates are reported in this dissertation as deviations from the mean of the deleted subclass. The subclass deleted was the one containing the largest number of observations. An analysis of variance utilizing the least squares estimates was performed on the data, using the method of fitting constants described in Appendix B.

As noted in Experiment I, only two transferrin alleles were present in the Hereford herd while three were found in the hybrid herd. Consequently, the data for each herd were analysed separately.

In the analysis of the data for the Hereford cows and 1962 Hereford calves, the effect of dehorning a number of heifers was confounded with the effect of source of dam, thus the effect of dehorning was not estimated in the linear model. Confounding also occurred between breed, source, and age of dam, so these factors were grouped into one termed breed group. Sire effect could not be analysed separately in the analysis of the 1961 calf records, and thus was included with breed, source and age of dam as breeding group.

D. Results

1. Analysis of 1961 Progeny Performance

Twenty-eight complete records were available for calves in the Hereford herd and 53 for calves in the hybrid herd. In the analysis of the records of the Hereford calves, breeding group included sire, and source and age of dam. In the analysis of the records of the calves in the hybrid herd, breeding group included breed, source and age of dam, sire being unknown except as to source.

Estimates of the effect of breeding group, sex and transferrin

Table 10

Means and least squares estimates of the effects of breeding group, sex and transferrin genotype on birth weight, calculated 180-day and 365-day weight for the 1961 Hereford calves

Classification		Symbol	Number of Records	Arithmetic Means			Least Squares Estimates(2)		
					Calculated			Calculated	
				Birth Weight	180-day Weight	365-day Weight	Birth Weight	180-day Weight	365-day Weight
			28	67.6	381.5	554.5	60.3466	399.4739	577.7325
Breeding Group (3)									
10K,	U.A., M	d_1	3	80.0	439.7	652.3	16.6023	68.0429	103.5631
UA 18P,	U.A., 2	d_2	5	65.6	365.0	563.0	3.1977	-11.0651	6.5904
UA 18P,	U.A., M	d_3	3	74.0	442.0	640.0	13.2783	53.6906	68.8434
M.C.I.,	M.R.C., 3	#d_4	8	63.4	383.4	539.2	0.0	0.0	0.0
Britisher,	Babey, 2	d_5	7	67.7	351.1	512.1	5.7181	-29.8797	-32.3431
Mischief,	Babey, 2	d_6	2	60.5	343.0	467.5	-1.6060	-16.9225	-27.4492
Sex									
Male		#s_1	16	67.6	395.0	589.8	0.0	0.0	0.0
Female		s_2	12	67.6	363.1	507.5	6.9028	-16.5631	-47.3414
Genotype									
Tf^A/Tf^A		g_1	3	58.3	321.3	439.7	-10.2868	-45.9768	-70.7036
Tf^D/Tf^D		g_2	7	71.3	381.7	588.8	1.1223	-33.4937	-19.7276
Tf^A/Tf^D		#g_4	18	67.7	391.4	560.3	0.0	0.0	0.0

# denotes the deleted variable

(2) Least squares estimates calculated as deviations from the deleted mean (See Apendix B)

(3) For a definition of Breeding Group see Appendix B and page 19 of text

Table 11

Means and least squares estimates of the effect of breeding group, sex and transferrin genotype on birth weight, calculated 180-day and 365-day weight for the 1961 calves in the hybrid herd

Classification	Symbol	Number of Records	Arithmetic Means			Least Squares Estimates(2)		
				Calculated			Calculated	
			Birth Weight	180-day Weight	365-day Weight	Birth Weight	180-day Weight	365-day Weight
	μ	53	66.4	409.8	586.3	61.6793	384.7030	500.5453
Breeding Group(3)								
Blades, Gal., 3	d_1	4	70.5	431.0	585.0	1.9471	30.1225	22.9932
A-7, Gal., 3	#d_2	22	65.5	391.2	534.9	0.0	0.0	0.0
Ohler, Char.X Ang., 2	d_3	8	67.8	440.2	604.5	2.1317	41.5673	56.2638
Bartsch, Ang., M	d_4	16	66.2	426.4	654.4	2.8271	33.7671	122.1188
U.A., Ang., M&2	d_5	3	63.3	349.3	553.7	-2.9895	-43.9351	10.2793
Sex								
Male	s_1	24	69.7	421.3	614.1	6.3985	21.4662	66.3660
Female	#s_2	29	63.6	400.4	563.3	0.0	0.0	0.0
Genotype								
Tf^A/Tf^A	g_1	10	66.7	392.7	590.6	1.1335	-17.2363	-4.2054
Tf^D/Tf^D	g_2	4	65.2	468.2	712.8	-2.2818	37.0967	73.3667
Tf^E/Tf^E	g_3	1	65.0	385.0	555.0	-3.0778	-21.1693	-11.9112
Tf^A/Tf^D	#g_4	22	65.4	409.7	580.3	0.0	0.0	0.0
Tf^D/Tf^E	g_5	8	70.0	425.4	605.9	3.4815	15.5041	42.8379
Tf^A/Tf^E	g_6	8	65.5	390.1	518.8	0.6680	-15.3544	-19.4638

# denotes the deleted variable

(2) Least squares estimates calculated as deviations from the deleted mean (See Appendix B)

(3) For definition of Breeding Group see Appendix B and page 19 of text. Gal. = Galloway, Char. = Charolais, Ang. = Angus

Table 12

Analysis of variance for the date in Table 10

Source of Variation	d.f.	Mean Squares: Calculated Birth Weight	Calculated 180-day Weight	Calculated 365-day Weight
Total	27	74.092	2515.666	5351.741
Breeding Group	5	145.989*	5349.056*	8731.095**
Sex	1	188.989*	1243.550	10,204.682*
Genotype	2	100.642	4000.198	5760.321
Error	19	42.894	1360.480	1864.862

* Significant at 5% level
**Significant at 1% level

Table 13

Analysis of variance for the data in Table 11

Source of Variation	d.f.	Mean Squares: Calculated Birth Weight	Calculated 180-day Weight	Calculated 365-day Weight
Total	52	41.498	2416.130	7025.799
Breeding Group	4	30.175	6335.078**	26,401.972**
Sex	1	454.410**	5036.268	48,157.875**
Genotype	5	21.200	2420.918	6732.482
Error	42	34.571	1755.449	3012.156

**Significant at the 1% level

genotype on birth weight, calculated 180-day weight and calculated 365-day weight were obtained. The subclass means and least squares estimates are summarized in Table 10 for the Hereford herd, and in Table 11 for the calves in the hybrid herd. Analysis of variance of the data represented by Tables 10 and 11 is shown in Tables 12 and 13, respectively.

Adjusted breeding group means differed for birth weight among the Hereford calves ($P < 0.05$), and for 180-day weight and 365-day weight for the calves in both herds ($P < 0.01$). Adjusted sex means differed for birth weight and 365-day weight in the Hereford herd ($P < 0.05$) and in the hybrid herd ($P < 0.01$). The adjusted genotype means for birth weight, 180-day weight and 365-day weight showed no significant difference in either herd ($P > 0.05$).

The error mean square for birth weight among the Hereford calves is somewhat larger than that among the calves in the hybrid herd, whereas the reverse situation exists for the 180-day and 365-day weights with the difference for 365-day weight being quite large. It appears that after major sources of variation (i.e. breed, source, sire, sex, etc.) are removed, there remains a greater variation in growth rate among calves in the hybrid herd than in the Hereford herd.

2. Analysis of 1962 Progeny Performance

Complete records were available for 52 and 62 calves in the Hereford and hybrid herds, respectively. For the Hereford calves, breed group refers to source and age of dam and to breed, source and age of dam for the calves in the hybrid herd.

Estimates of the effect of sire, breed group, sex, and transferrin genotype were obtained for birth weight and calculated 180-day weight. The subclass means and least squares estimates are summarized in Table 14

for the Hereford calves, and in Table 15 for the hybrid calves. Analysis of variance of the data represented by Tables 14 and 15 is shown in Tables 16 and 17, respectively. During the least squares estimation of the sums of squares for birth weight due to sires, in the hybrid herd, it was found that the matrix involved was ill-conditioned. Consequently, a suitable inversion was not obtained, making statistical analysis of this parameter not reliable.

The adjusted sire means for birth weight of the Hereford calves differed significantly ($P < 0.01$). Least squares estimates for the same means for the hybrid calves showed differences, but as indicated above these could not be tested statistically. Differences were found among the adjusted breed group means for birth weight and 180-day weight of the Hereford calves, and for 180-day weight of the hybrid calves ($P < 0.01$). There was no significant difference ($P > 0.05$) among the adjusted breed group means for birth weight of the hybrid calves. No significant sex difference was found in the Hereford calves ($P > 0.05$), although male calves were 3.0 lb. heavier than females at birth and females were 15.2 lb. heavier than males at 180 days. Male hybrid calves were 9.0 lb. heavier than females at birth and 33.1 lb. heavier at 180 days ($P < 0.01$). There were no significant differences among adjusted genotype means for birth weight or 180-day weight of the Hereford calves, nor for birth weight of the hybrid calves. There were however, differences among the adjusted genotype means for 180-day weight of the hybrid calves ($P < 0.05$). However, it is questionable whether this effect is real since a major portion of the variation was due to genotype Tf^{A}/Tf^{A}, a subclass represented by only three individuals.

The error mean square for birth weight among the hybrid calves was

Table 14

Means and least squares estimates of the effect of sire, breed group, sex and transferrin genotype on birth weight and calculated 180-day weight for the 1962 Hereford calves

Classification	Symbol	Number of Records	Means		Estimates(2)	
			Birth Weight	Calculated 180-day Weight	Birth Weight	Calculated 180-day Weight
	μ	52	70.4	328.3	70.6357	366.4309
Sire						
UA 8R	#a_1	25	69.4	330.4	0.0	0.0
UA 11P	a_2	18	67.7	320.7	-3.2177	-17.3573
FXE 28R	a_3	9	78.7	338.7	10.6109	4.5040
Breed Group(3)						
UA 2	b_1	5	63.2	250.4	-10.5611	-82.7933
UA 3	b_2	4	58.8	324.2	-16.4115	-18.0436
UA 4 & older	b_3	2	84.0	453.0	9.2435	125.0185
M.R.C. 3	#b_4	13	74.6	337.7	0.0	0.0
M.R.C. 4	b_5	8	74.8	387.5	-0.1090	50.9620
Babey 3	b_6	9	68.7	336.7	-5.5222	-4.1246
Powlesland 3	b_7	4	73.0	230.5	-3.7070	-110.5445
Porteous 2	b_8	7	66.1	310.4	-8.8599	-23.6452
Sex						
Males	s_1	25	71.4	315.5	3.0133	-15.1954
Females	#s_2	27	69.4	340.1	0.0	0.0
Genotype						
Tf^A/Tf^A	g_1	16	70.5	334.9	2.2639	20.6503
Tf^D/Tf^D	g_2	12	73.1	343.1	5.4328	30.8493
Tf^A/Tf^D	#g_4	24	69.0	316.5	0.0	0.0

# denotes the deleted variable

(2) Least squares estimates calculated as deviations from the deleted mean (See Appendix B)

(3) For definition of Breed Group see Appendix B

Table 15

Means and least squares estimates of the effect of sire, breed group, sex and transferrin genotype on birth weight and calculated 180-day weight for the 1962 hybrid calves

Classification	Symbol	Number of Records	Arithmetic Means: Birth Weight	Arithmetic Means: Calculated 180-day Weight	Least Squares Estimates[2]: Birth Weight	Least Squares Estimates[2]: Calculated 180-day Weight
	μ	62	73.6	412.9	71.9572	398.9758
Sire						
Evador, Ang.	a_1	6	68.7	415.5	-7.2211	22.4914
Rex Dun, Gal.	a_2	8	58.2	323.8	-14.0285	-20.1647
UA Dun, Gal.	a_3	8	66.8	415.5	-9.1222	25.6690
UA 7R, Ang.	a_4	16	78.0	415.5	0.0	0.0
Etienne, Char.	a_5	11	82.8	454.3	9.5513	55.6073
Sir Alto, Char.	a_6	9	79.2	433.2	3.9041	22.6218
AW 5R, Gal.	a_7	4	69.8	411.8	-0.3606	2.5400
Breed Group[3]						
UA, Ang. 2	b_1	5	55.0	267.4	-12.6468	-146.5678
UA, Ang. 4 +	b_2	3	81.0	405.0	-3.5915	-39.9251
Glenbow, Gal. 3	b_3	19	74.3	437.2	-4.1080	1.9502
A-7, Gal. 4	#b_4	23	76.5	418.2	0.0	0.0
Blades, Gal. 4	b_5	5	81.6	445.2	7.1387	28.6475
Ohler, Char. X Ang. 3	b_6	7	66.6	413.7	0.1070	-4.3263
Sex						
Male	s_1	26	77.8	427.5	9.0454	33.1361
Female	s_2	36	70.5	402.3	0.0	0.0
Genotype						
Tf^A/Tf^A	g_1	3	59.3	359.3	5.1496	53.3543
Tf^D/Tf^D	g_2	15	80.9	431.3	1.6508	-0.8803
Tf^E/Tf^E	g_3	None	--	--	--	--
Tf^A/Tf^D	g_4	25	75.1	433.0	0.0	0.0
Tf^D/Tf^E	g_5	12	68.8	395.8	1.1506	-21.4294
Tf^A/Tf^E	g_6	7	66.9	353.9	2.5491	-27.3639

# denotes the deleted variable

(2) Least squares estimates calculated as deviations from the deleted mean (See Appendix B)

(3) For definition of Breed Group grouping see Appendix B

Table 16

Analysis of variance for the data in Table 14

Source of Variation	d.f.	Mean Squares: Birth Weight	Mean Squares: Calculated 180-day Weight
Total	51	127.378	4997.729
Sires	2	475.681**	1833.287
Breed Group	7	301.025**	19,198.797**
Sex	1	48.150	2374.796
Genotypes	2	107.547	2004.424
Error	39	66.425	2713.828

**Significant at 5% level

Table 17

Analysis of variance for the data in Table 15

Source of Variation	d.f.	Mean Squares: Birth Weight	Mean Squares: Calculated 180-day Weight
Total	61	149.261	3931.000
Sire	6	--- (1)	3777.233*
Breed Group	5	112.854	9699.209**
Sex	1	980.478**	12,364.468**
Genotype	4	14.127	3569.113*
Error	45	75.645	1339.981

* Significant at 5% level
**Significant at 1% level
(1)Not estimated

somewhat larger than that for the Hereford calves, whereas the reverse situation existed for 180-day weight. The results of this analysis contrast with those of the analysis of the 1961 records, in which the error mean square for birth weight was slightly higher for the Hereford calves than for the calves in the hybrid herd. The error mean squares for 180-day weight were also of reverse rank in the two analyses.

3. Analysis of the Performance of the Cows during 1961 and 1962

Complete records were available for 75 Hereford cows and 76 cows in the hybrid herd. Breed group is the same as that defined in section (2) above. Estimates of the effect of breed group, transferrin genotype, and pregnancy were obtained on accumulated weight loss over three periods during the fall of 1961 and spring of 1962, and on the standard weight of the cows. The subclass means and least squares estimates are summarized in Table 18 for the Hereford cows, and in Table 19 for the cows in the hybrid herd. Analysis of variance of the data represented by Tables 18 and 19 is shown in Tables 20 and 21, respectively.

Significant differences ($P < 0.01$) were observed among adjusted breed group means for weight loss of the cows in both herds, except for the period from August to June for the cows in the hybrid herd. The breed groups differed during this period at $P < 0.05$. The corresponding genotype means did not differ significantly ($P > 0.05$) in either herd, although examination of the least squares estimates of the effect of genotype suggests that differences may have been present but were masked by the high variation in the population.

Pregnancy had a significant effect on accumulated weight loss over the periods studied which corresponded to later stages of gestation. Thus,

Table 18

Means and least squares estimates of the effect of breed group, transferrin genotype and pregnancy on winter weight loss and standard cow weight for the Hereford cows during 1961 and 1962

Classification	Symbol	Number of Records	Weight loss from August 29th to: Feb. 1	April 6	June 26	Standard Cow Weight
A. ARITHMETIC MEANS						
	μ	75	130.0	98.8	93.1	966.4
Breed Group(3)						
U.A., 2	b_1	8	113.8	89.4	67.5	858.5
U.A., 3	b_2	9	156.1	134.4	95.6	941.6
U.A., M	b_3	4	320.0	272.5	296.5	1291.2
M.R.C., 3	b_4	13	98.8	72.3	79.6	1065.0
M.R.C., 4	b_5	10	95.0	61.0	70.0	965.6
Babey, 3	b_6	10	130.0	84.0	83.0	877.8
Powlesland, 3	b_7	8	141.2	119.4	71.2	1047.6
Porteous, 2	#b_8	13	114.6	80.8	96.9	870.4
Genotype						
Tf^A/Tf^A	g_1	14	104.6	83.6	104.6	895.2
Tf^D/Tf^D	g_2	19	137.9	106.6	90.0	968.2
Tf^A/Tf^D	#g_4	42	134.9	100.4	90.6	989.4
Pregnancy						
Pregnant(4)	#p_1	58	117.3	82.0	111.3	952.8
Open	p_2	17	173.2	157.4	30.9	1012.9
B. LEAST SQUARES ESTIMATES(2)						
	μ	75	105.9656	65.2838	103.3879	846.3249
Breed Group(3)						
U.A., 2	b_1	8	3.2912	3.8104	0.0612	-9.1287
U.A., 3	b_2	9	37.8083	39.8651	30.7729	58.8334
U.A., M	b_3	4	201.3754	182.7068	253.0759	422.3653
M.R.C., 3	b_4	13	-12.2242	1.6501	-29.9852	207.1854
M.R.C., 4	b_5	10	-11.3832	-10.7805	-26.9308	111.1803
Babey, 3	b_6	10	23.8444	10.5488	-17.7332	19.7218
Powlesland, 3	b_7	8	22.9099	27.4934	23.3229	174.1672
Porteous, 2	b_8	13	0.0	0.0	0.0	0.0
Genotype						
Tf^A/Tf^A	g_1	14	-18.8664	-0.7583	17.8329	-0.7567
Tf^D/Tf^D	g_2	19	16.5906	17.4648	20.1910	37.3416
Tf^A/Tf^D	#g_3	42	0.0	0.0	0.0	0.0
Pregnancy						
Pregnant(4)	#p	58	0.0	0.0	0.0	0.0
Open	p	17	25.3180	49.0188	-120.4276	45.1196

# denotes the deleted variable

(2) Least squares estimates calculated as deviations from the deleted mean (See Appendix B)

(3) For a definition of Breed Group see Appendix B

(4) Defined as giving birth to a calf in spring 1962

Table 19

Means and least squares estimates of the effect of breed group, transferrin genotype and pregnancy on winter weight loss and standard cow weight for the cows in the hybrid herd during 1961 and 1962

Classification	Symbol	Number of Records	Weight loss from August 29th to: Feb. 1	April 6	June 26	Standard Cow Weight
A. ARITHMETIC MEANS						
		76	92.7	51.4	91.6	954.0
Breed Group(3)						
U.A., Ang. 2	b_1	8	95.0	57.5	37.5	777.0
U.A., Ang. M	b_2	4	185.0	146.2	186.2	1060.5
Glenbow, Ang. 3	b_3	19	57.4	26.3	119.2	946.5
A-7, Gal. 4	#b_4	26	109.0	50.6	98.5	968.2
Blades, Gal. 4	b_5	9	110.6	73.3	82.2	1071.8
Ohler, Ch.XAng. 3	b_6	10	62.5	37.0	35.0	924.0
Genotype						
Tf^A/Tf^A	g_1	9	108.3	68.9	69.4	923.2
Tf^D/Tf^D	g_2	17	66.5	32.4	80.6	954.6
Tf^E/Tf^E	g_3	1	115	75	75	930
Tf^A/Tf^D	#g_4	34	98.7	61.9	96.9	969.6
Tf^D/Tf^E	g_5	9	128.3	63.9	126.1	919.8
Tf^A/Tf^E	g_6	6	52.5	-2.5	76.7	964.5
Pregnancy						
Pregnant(4)	#p_1	65	90.5	45.3	114.1	951.5
Open	p_2	11	105.4	87.7	-41.4	968.6
B. LEAST SQUARES ESTIMATES(2)						
		76	107.9984	51.6281	125.2833	989.3020
Breed Group(3)						
U.A., Ang., 2	b_1	8	-13.6238	-0.9186	-42.9439	-208.0485
U.A., Ang., M	b_2	4	74.0803	86.3078	102.1102	74.9564
Glenbow, Ang., 3	b_3	19	-42.8174	-15.8729	10.5836	-34.5782
A-7, Gal., 4	#b_4	26	0.0	0.0	0.0	0.0
Blades, Gal., 4	b_5	9	2.4573	12.3978	13.9780	82.0115
Ohler, Ch.XAng. 3	b_6	10	-43.0658	-20.9838	-30.3803	-58.6146
Genotype						
Tf^A/Tf^A	g_1	9	7.8949	1.9066	-4.9690	-26.6196
Tf^D/Tf^D	g_2	17	-18.3675	-12.1886	-29.5670	-3.9607
Tf^E/Tf^E	g_3	1	7.0016	23.3719	-50.2833	-59.3020
Tf^A/Tf^D	#g_4	34	0.0	0.0	0.0	0.0
Tf^D/Tf^E	g_5	9	29.8775	16.3560	3.2752	-59.1695
Tf^A/Tf^E	g_6	6	-34.0897	-46.1916	-53.9084	-7.5129
Pregnancy						
Pregnant(4)	#p_1	65	0.0	0.0	0.0	0.0
Open	p_2	11	3.7902	31.3498	-159.6051	11.5861

# denotes the deleted variable

(2) Least squares estimates calculated as deviations from the deleted mean (See Appendix B)

(3) For a definition of Breed Group see Appendix B

(4) Defined as giving birth to a calf in spring 1962

Table 20

Analysis of variance for the data in Table 18

Source of Variation	d.f.	Mean Squares: Weight loss from August 29 to: Feb. 1	April 6	June 26	Standard Cow Weight
Total	74	4237.84	4047.19	7292.50	1591.53
Breed Group	7	21,677.43**	14,638.24**	37,016.37**	115,871.25**
Genotype	2	4507.34	1931.10	4870.49	8708.82
Pregnancy	1	6576.38	24,652.31**	108,685.10**	20,778.65*
Error	64	1774.31	1594.36	3048.33	3087.30

* Significant at the 5% level
**Significant at the 1% level

Table 21

Analysis of variance for the data in Table 19

Source of Variation	d.f.	Mean Squares: Weight loss from August 29 to: Feb. 1	April 6	June 26	Standard Cow Weight
Total	75	3710.96	3537.88	7476.14	11,601.16
Breed Group	5	10,509.04**	14,412.88**	11,568.90*	121,665.86**
Genotype	5	4006.67	3120.30	4526.67	5267.13
Pregnancy	1	10,109.98	7486.47	195,492.92**	1029.90
Error	64	2817.01	2742.94	3673.45	6343.56

* Significant at the 5% level
**Significant at the 1% level

in the Hereford herd, open cows lost 49.0 lb. more weight than pregnant cows over the period from August to April, but lost 120.4 lb. less than pregnant cows over the period from August to June ($P < 0.01$). Although not significant ($P > 0.05$), open cows in the hybrid herd lost 31.4 more weight than pregnant cows over the period of August to April. As in the Hereford herd, open cows in the hybrid herd lost 159.6 lb. less weight than pregnant cows over the period of August to June ($P < 0.01$). The apparent advantage of pregnant cows in April weights reflects gain during gestation and the subsequent June weights reflect losses due to calving and nursing.

The breed groups of both herds differed ($P < 0.01$) in standard weight of the cows. In the Hereford herd, pregnancy also affected standard weight ($P < 0.05$), the open cows being 45.1 lb. heavier than pregnant cows. There was a large difference in the error mean squares for standard weight in the two herds, that of the hybrid herd being of the order of two times that of the Hereford herd. This statistic illustrates the great variation which exists among the foundation females of the hybrid herd.

4. Analysis of the Effect of Genotype of Dam on Performance of 1962 Progeny

Complete records were available for 47 Hereford cows and calves and for 59 cows and calves in the hybrid herd. Breed group in the analysis of the Hereford records refers only to source, and in the hybrid herd refers to breed and source. Estimates of the effect of breed group and transferrin genotype of the dam, and sex of the calf were obtained on birth weight and calculated 180-day weight of the calf. The subclass means and least squares estimates are summarized in Table 22 for the Hereford data,

and in Table 23 for the data of the hybrid herd. Analysis of variance of the data is presented in Tables 24 and 25, respectively.

No differences were observed among adjusted genotype of dam means for birth weight and 180-day weight of the calves in the Hereford herd ($P > 0.05$). The same means for birth weight of the calves in the hybrid herd differed ($P < 0.01$), however a major portion of the variation was due to genotype Tf^E/Tf^E, a subclass represented by only one individual. No differences were apparent for genotype of dam means for 180-day weight of the hybrid calves ($P > 0.05$). The effect of dehorning was also estimated in the analysis of the Hereford data, but although calves from dams not dehorned were estimated as 23.8 lb. heavier at 180 days, the difference was not significant ($P > 0.05$).

In this analysis no significant differences ($P > 0.05$) were observed among adjusted breed group means for birth weight and 180-day weight of Hereford calves, whereas differences ($P < 0.01$) were observed in the analysis of the 1962 progeny performance (Table 16, section 2). The linear model for the analysis in this section differed from that used in section 2 in two ways; sire was not included in the model and the age factor was not included in breed group. Sire differences ($P < 0.01$) were found in the analysis of birth weight in section 2, so that the error mean square for birth weight in the above analysis of effect of dam's genotype was larger than necessary. However, no sire effect was observed for 180-day weight in section 2 so the error mean square for 180-day weight in the above analysis was not greatly in error. Thus, it would appear that since no differences were observed in the breed group means for 180-day weight in the above analysis, age difference was the factor causing the major portion of the variation observed in the previous analysis.

Table 22

Means and least squares estimates of the effect of breed group, transferrin genotype and dehorning of the cows and the effect of sex of calf on birth weight and calculated 180-day weight for the 1962 Hereford calves

Classification	Symbol	Number of Records	Arithmetic Means: Birth Weight	Arithmetic Means: Calculated 180-day Weight	Least Squares Estimates[2]: Birth Weight	Least Squares Estimates[2]: Calculated 180-day Weights
	μ	47	70.1	328.6	72.8017	357.7306
Breed Group[3]						
U.A.	b_1	11	65.4	314.1	-10.5305	-47.7363
M.R.C.	$\#b_2$	18	74.7	358.7	0.0	0.0
Babey	b_3	9	68.7	336.7	-5.6471	-12.1089
Powlesland	b_4	4	73.0	230.5	-0.5166	-117.3469
Porteous	b_5	5	64.0	316.0	-9.6576	-33.3265
Genotype						
Tf^A/Tf^A	g_1	9	69.7	325.7	1.4198	-2.4858
Tf^D/Tf^D	g_2	8	65.1	308.1	-3.6307	-5.9310
Tf^A/Tf^D	$\#g_4$	30	71.5	334.9	0.0	0.0
Dehorning						
Dehorned	$\#h_1$	32	69.5	320.9	0.0	0.0
Not dehorned	h_2	15	71.3	344.9	2.0643	23.8016
Sex (Calf)						
Males	s_1	23	71.5	318.8	3.2451	-16.8018
Females	$\#s_2$	24	68.7	338.0	0.0	0.0

# denotes the deleted variable

(2) Least squares estimates calculated as deviations from the deleted mean (See Appendix B)

(3) For a definition of Breed Group see Appendix B

Table 23

Means and least squares estimates of the effect of breed group and transferrin genotype of the cow and the effect of sex of calf on birth weight and calculated 180-day weight for 1962 hybrid calves

Classification	Symbol	Number of Records	Arithmetic Means: Birth Weight	Arithmetic Means: Calculated 180-day Weight	Least Squares Estimates(2): Birth Weight	Least Squares Estimates(2): Calculated 180-day Weights
μ		59	73.2	404.2	72.1421	375.8853
Breed Group(3)						
U.A., Ang.	b_1	8	64.8	319.0	-12.2378	-76.1763
Glenbow, Ang.	b_2	17	73.8	433.8	-3.4040	39.3891
A-7, Gal.	#b_3	21	79.7	418.8	0.0	0.0
Blade, Gal.	b_4	5	81.6	445.2	6.2947	53.5829
Ohler, Ch.XAng.	b_5	7	66.6	413.7	-13.9704	12.6429
Genotype						
Tf^A/Tf^A	g_1	6	71.7	394.5	0.3619	2.9407
Tf^D/Tf^D	g_2	12	77.3	408.9	7.4950	-0.0490
Tf^E/Tf^E	g_3	1	83	389	10.8579	13.1147
Tf^A/Tf^D	#g_4	25	74.6	413.0	0.0	0.0
Tf^D/Tf^E	g_5	9	71.6	423.6	-2.0903	25.0627
Tf^A/Tf^E	g_6	5	74.2	409.0	1.8741	9.7873
Sex (calf)						
Males	s_1	25	77.1	423.4	7.7272	37.8590
Females	#s_2	33	72.5	401.8	0.0	0.0

# denotes the deleted variable

(2) Least squares estimates calculated as deviations from the deleted mean (See Appendix B)

(3) For the definition of Breed Group see Appendix B

Table 24

Analysis of variance for the data in Table 22

Source of Variation	d.f.	Mean Squares: Birth Weight	Mean Squares: Calculated 180-day Weight
Total	46	117.08	5411.42
Breed Group	4	202.20	1158.64
Genotype	2	52.38	98.58
Dehorning	1	24.91	3291.35
Sex	1	116.44	13,122.99
Error	38	110.37	4826.29

Table 25

Analysis of variance for the data in Table 23

Source of Variation	d.f.	Mean Squares: Birth Weight	Mean Squares: Calculated 180-day Weight
Total	58	141.98	7097.34
Breed Group	4	382.95*	21,063.94*
Genotype	5	894.22**	997.58
Sex (calf)	1	786.09*	870.85
Error	48	109.76	6366.30

* Significant at the 5% level
**Significant at the 1% level

E. Discussion

Briles (1960) in a review of blood groups in chickens, suggested that blood group genes were in some way associated with physiological effects related to survival and that some selective mechanism favoring heterozygotes over homozygotes was operative. Similar effects of blood groups in cattle have been discussed by Robertson (1961), although little supporting evidence has been found. Ashton (1960b) studying the transferrin locus in cattle reported finding a selective advantage for the Tf^D allele in milk yield. Ashton (1961) also found that matings involving individuals homozygous at the transferrin locus were more fertile than matings involving heterozygotes.

The results reported in this study do not show any differences among the genotypes with regard to the traits studied, although the least squares estimates suggest a possible superiority of the Tf^A/Tf^D genotype. Briles and Allen (1961) in discussing B blood group alleles in chickens suggested that in the evaluation of quantitative traits we are dealing at most with only the periphery of many physiological processes as they are integrated over the life span of an individual. Consequently, the magnitude of the differences in the measurable effects of various genotypes at a single locus would usually be expected to be small, and exceptionally large samples would be required to establish their existence with desirable levels of confidence. Nevertheless, such differences if they existed would be biologically important, especially at the population level. Such would be the case in studies of production traits in beef cattle. It is evident from the gene frequencies of the transferrin alleles that some force or mechanism is operating to maintain a stable polymorphism. The stability of the polymorphism is illustrated by the extremely low frequency

of the Tf^E allele maintained in some breeds and the relatively high levels maintained in others. Similar unaccountable gene frequency values for the ABO blood group system of man are discussed by Briles, et al. (1957).

Since the transferrins are physiologically active proteins of the blood, a selective advantage of one genotype over another could conceivably be a possibility. Maintenance of the less advantageous genes in the population could result from selection advantage being in favor of the heterozygotes or could result from pleiotropic effects of the alleles. The data available to date do not exclude the possibility that the alleles are neutral in effect and that recurrent mutation balanced in two or more directions maintains a stable polymorphism.

The effects of breed, source and age of dam are confounded in the present analysis. However, the breed group differences found in the majority of the analyses illustrate that variation exists in the two herds studied. Although age appears to be the major factor in the results reported herein, an analysis of the breed and source effects suggested that differences exist among the breeds in the hybrid herd and among the sources as represented by the herds samples (R.T. Berg, unpublished data). The sire differences found in the analysis of the performance of the 1962 calves are consistent with general results that sires do influence the performance of their progeny to a considerable degree.

F. Summary

1. Data collected at the University Ranch in the performance testing program were analysed for the effects of sire, sex, breed, source and age of dam and transferrin genotype on several production traits. Data were available for two calf crops, 1961 and 1962, from two herds, a Hereford herd and a hybrid herd. The performance of the cows during the winter of

1961-62 were also studied.

2. Breed group effects on birth weight and calculated 180-day weight were found for both 1961 and 1962 Hereford calves, and on calculated 180-day weight for the 1961 and 1962 calves in the hybrid herd. No breed group effect on birth weight was found for the calves in the hybrid herd. Breed group effects were also found on calculated 365-day weight for the records of 1961 calves in both herds.

3. Sire effects were found for birth weight in the analysis of the 1962 Hereford records and for calculated 180-day weight in the 1962 hybrid records.

4. Sex effects were found for birth weight and 365-day weight of the calves in the 1961 Hereford and hybrid herds, and for birth weight and 180-day weight of the calves in the 1962 hybrid herd. No significant sex effects were found in the analysis of the 1962 Hereford records, although Hereford females were estimated as 15 lb. heavier than males at 180-days.

5. Differences due to effects of breed group and pregnancy were found among the performance of cows during the winter of 1961 and spring of 1962. Pregnancy was found to offset weight loss during the winter and calving and nursing were found to have a similar effect on expected weight gain during the spring. Breed group was also found to affect the standard weight of the cows in both herds. Open Hereford cows were found to be 45.1 lb. heavier than pregnant and nursing Hereford cows when estimated as standard cow weight.

6. No statistical relation was found between transferrin genotype and weight at birth or at 180 days for calves in either the Hereford or hybrid herds at the University of Alberta Ranch. However, the results of the analyses indicate a possible advantage for the Tf^{A}/Tf^{D} genotype in

relation to some of the traits studied. In a similar analysis of accumulated weight loss of cows in both herds during the winter of 1961-62, no statistical relation was found between transferrin genotype and this trait, although the Tf^{A}/Tf^{D} genotype appeared to have an advantage over some other genotypes.

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APPENDIX A - LABORATORY PROCEDURES

A. Collection of Blood

Blood samples were collected from the external jugular vein, using a 3 inch, No. 15 bleeding needle, held in a needle holder (B-D No. 510 Blood Taking Needle Holder). The blood was received, in such a way as to prevent foaming, into a clean dry 15 x 125 mm. test tube supported by the needle holder. The tube was allowed to fill to a level of one-half, was stoppered with a No. 3 cork, and placed in a horizontal position so that contact occurred between the blood sample and the cork. The sample was left at air temperature for 1/2 to 1 hour to facilitate clotting, after which time it was placed in an insulated picnic chest containing crushed ice. The blood was transported in this manner to the Animal Science laboratories in Edmonton. Care was taken at all times to avoid hemolysis.

B. Preparation of Sera

On arrival at the laboratory the blood samples were placed in a refrigerator at 5^{o}C. overnight to allow clot shrinkage to occur. It was sometimes found necessary to break the clot away from the sides of the tube by gently tapping the tubes before placing them in the refrigerator. The tubes were kept in a horizontal position at all times to cause the clot to shrink away from the sides of the tube and toward the cork.

The following morning the samples were removed from the refrigerator. The cork was removed carefully in order to withdraw the clot adhered to the cork. Handling the sample in this manner made it possible to remove a large portion of the red cells without centrifugation. The fluid remaining was transferred to a clean tube and centrifuged for 5 minutes at 775 g in a refrigerated angle centrifuge. The serum was decanted into 2 ml. plastic semen vials and stored at - 10^{o}C.

C. Preparation of Starch Gels

(a) Gel composition

The starch gels were prepared with 65 gm. (13 gm./100 ml.) hydrolysed starch (Connaught Medical Laboratories, Toronto) in 500 ml. of borate buffer of pH 8.65. The buffer was prepared by dissolving 1.61 gm. of boric acid and 0.36 gm. of sodium hydroxide solution in 1000 ml. of distilled water and adding 1 N sodium hydroxide solution until the desired pH was obtained. The resulting buffer concentration was 0.026 M borate with an ionic strength of approximately 0.1.

(b) Gel preparation (Kristjansson, 1960b)

The hydrolysed starch was suspended in 125 ml. of cold buffer solution in a 1000 ml. Erlenmeyer flask. The remaining 375 ml. of buffer solution was heated in a narrow necked 500 ml. Florence flask to a termperature of 95°C. The flask containing the hot buffer solution was then inverted into the flask containing the starch suspension, rotating both to provide gradual addition of the hot buffer. After all the hot buffer had been added, the Erlenmeyer was shaken vigorously to produce a homogeneous mixture. The starch solution was de-gassed by applying a negative pressure of 27 lb. with an aspirator attached to the water line, until the contents boiled vigorously. The time of boiling was kept as short as possible (10 - 15 sec.) to prevent water loss. The starch solution was then poured into the gel trays.

D. Preparation of Bridge Solution

The bridge solution was prepared by dissolving 18.55 gm. of boric acid and 2.40 gm. of sodium hydroxide per liter and adjusting the pH to

8.45 by the addition of 1 N sodium hydroxide. The resulting solution was approximately 0.3 M borate. The concentration of the bridge solution has a pronounced influence on the anodic end of the starch gel during electrophoresis. If the bridge solution is too dilute it will cause a swelling of the gel and if the solution is too concentrated it will cause the gel to shrink.

E. Electrophoretic Procedure[1]

Four gel trays[2], with end plates in position, were placed horizontally on absorbent paper. The covers for the trays were lightly oiled with mineral oil and placed in an over at 70°C.

The hot starch solution (freshly prepared) was poured into the gel tray and the hot cover immediately lowered into position. Air bubbles were avoided by lowering the cover from one side, thus forcing the excess starch solution across the tray. The cover was weighted with six 400 ml. beakers each containing approximately 300 ml. of water, and left for 1-1/2 to 2 hours to allow the gel to cool. Air trapped between the sample slots was forced in the retrograde direction by placing the weights next to the sample slots first, and radiating them to the edges.

When the gels had cooled sufficiently, the covers were removed. Disruption of the sample slots was avoided by placing one hand over the slot former and applying pressure while at the same time lifting the cover from the distal end. When the cover was free of the gel, the pressure on the slot former was released allowing the cover to lift from the region of the sample slots.

The serum samples (approx. 0.05 ml.) were inserted into the sample slots with a pasture pipette, duplicating the two outer samples to exclude

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1 The electrophoretic procedure outlined here is that of Smithies, 1955, except for some modification and is presented for completeness

2 Equipment was that supplied for "Vertical Starch-Gel Electrophoresis, (according to Smithies, 1959b)" Otto Hiller, Madison, Wisconsin

edge effects. In this way, six samples were run per gel. Petroleum jelly, previously heated to 45°C, was poured directly over the region of the sample slots. The end plates were then removed, the edges of the gel trimmed, and the remainder of the exposed gel, except the ends, covered with polyethylene film (Saran Wrap). The gel trays were then assembled as outlined by Smithies (1959a) except that platinum electrodes were used (Fig. 2). A voltage gradient of 7 v/cm. d.c. (225 v/gel) was applied and process allowed to run for 10-1/2 hours. The power supply used was "Heathkit Variable Voltage Regulated Power Supply, Model Ps-3" (The Heath Company, Benton Harbor, Michigan). The voltage gradient across the gels was measured directly with a high resistance voltmeter (20,000 ohms/volt) using probes inserted into the ends of the gels.

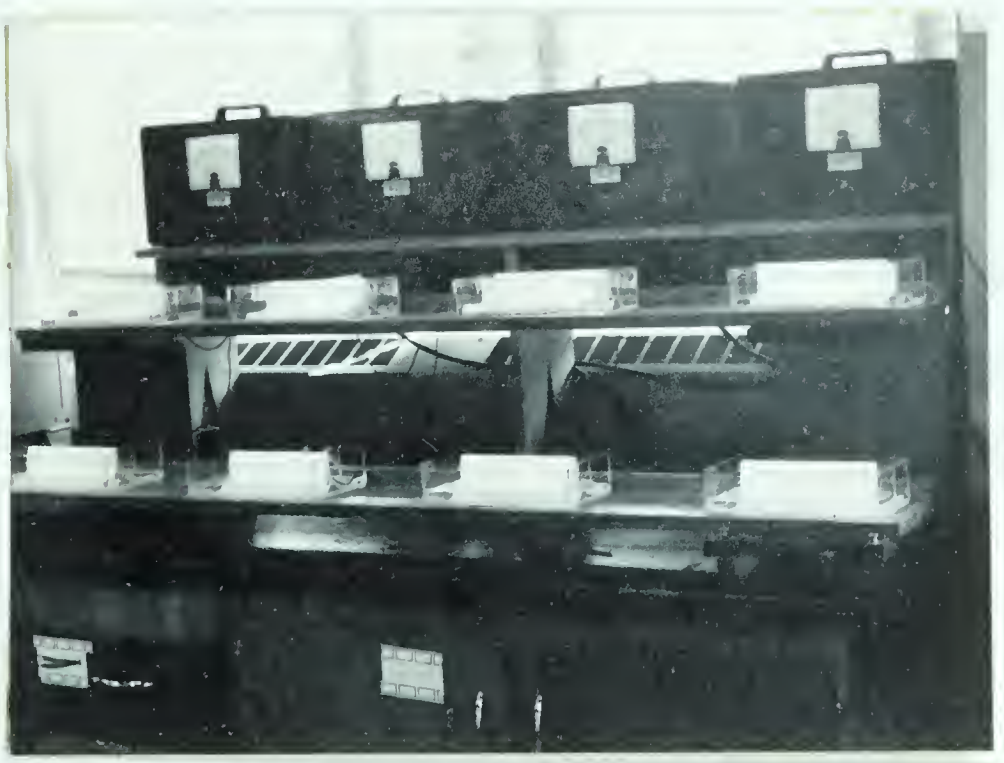

Fig. 5 Photograph showing the arrangement of the power supplies and bridge solution chambers prior to electrophoresis.

After electrophoresis was completed the trays were dismantled and placed in the refrigerator to cool. The gels were then sliced according to the method of Smithies (1959a) and stained for protein detection with Amido-Black 10 B as outlined by Beaton, et al. (1961) except that methanol/water/acetic acid in the proportions 50:50:10 v/v was used as the dye solvent throughout.

The gels were destained with a mixture of DEAE-cellulose and solka floc (SW40A) as the dye absorbent in an "Electrophoretic Starch-gel Destainer" (Otto Hiller, Madison, Wisconsin).

The staining procedure used for iron detection was that of Beaton, et al. (1961).

F. Photography of Gels

Gels stained with Amido-Black 10B were allowed to "age" for 12 to 24 hours in a tray containing dye solution, to obtain a more rigid gel.

Gels were photographed on Kodak 35 mm Panatomic-X, fine grain, black and white film with tungsten-lamp illumination. The gels were placed in a white enamel tray containing sufficient dye solvent to completely cover the gel. Two No. 2 photoflood lamps were placed at the same height as the mounted camera, and on a line parallel to the camera and which intersected the lens of the camera. The lamps were set such that the light beam from each crossed in space below the camera lens and struck the surface of the liquid on the opposite side at an angle of 45 degrees (Figure 3). The film was developed commercially.

The gels were wrapped separately in polyethylene film and stored in a refrigerator at 5°C. No appreciable fading occurred after four months of storage.

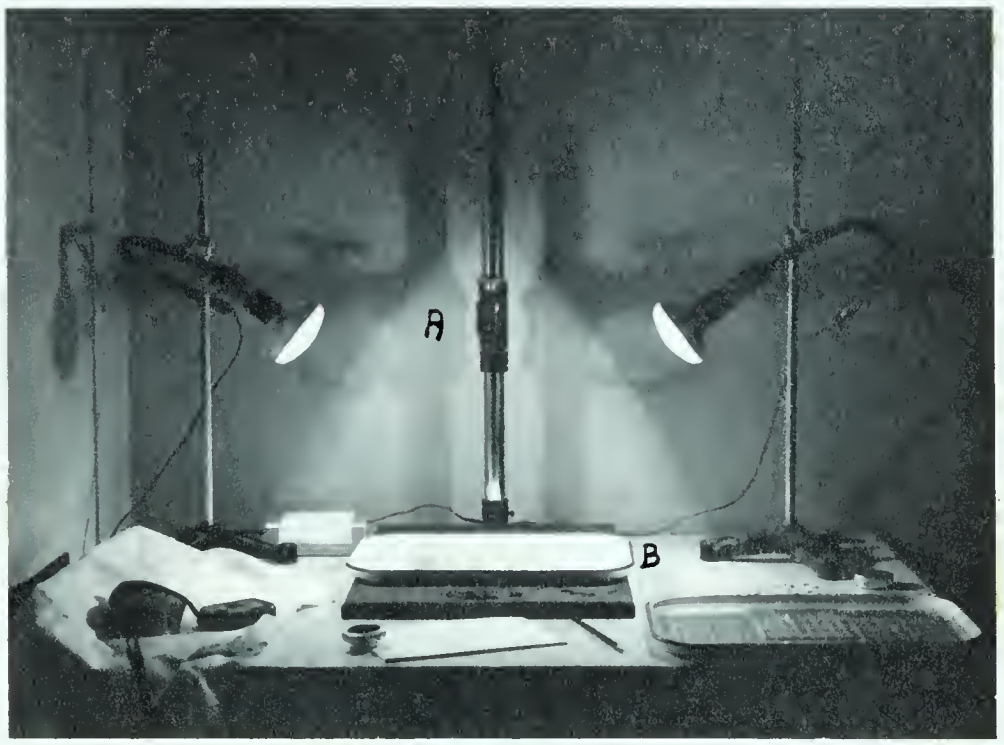

Fig. 6 Photograph showing the arrangement of the photoflood lamps and camera stand used for photographing the starch gels. A. Position of the camera. B. Tray containing dye solvent and the gel to be photographed.

APPENDIX B - STATISTICAL ANALYSIS

A. Method of Statistical Analysis

The object of the analysis was to obtain unbiased estimates of certain parameters and to perform an analysis of variance on these parameters. The estimates were obtained by the method of least squares and the analysis of variance was performed using the method of fitting constants (Kempthorne, 1952).

Assuming no interactions, mathematical models of the following general form were organized:

$$Y_{ijk..} = \mu + a_i + b_j + c_k + \ldots + e_{ijk..}$$

where: $i = 1,2,\ldots m$; $j = 1,2,\ldots n$; $k = 1,2,\ldots p$; where μ, a_i, b_j and c_k are the parameters and $(\mu + a_i + b_j + c_k + \ldots)$ is the expected value of $Y_{ijk..}$, when $Y_{ijk..}$ is the yield Y of the $ijk..^{th}$ individual; where the $e_{ijk..}$'s are normally and independently distributed around a mean of zero with variance σ^2; and where $n_{ijk..}$ is the number of observations in the $ijk..^{th}$ cell. The parameters are defined as:

u = the best estimate of the population mean;

a_i = the effect of the i^{th} factor of the A classification;

b_j = the effect of the j^{th} factor of the B classification;

c_k = the effect of the k^{th} factor of the C classification.

Based on the mathematical model, simultaneous equations were organized in the form of an r x r matrix with s right hand sides. The equation which contained the largest number of observations was deleted from each classification, i.e. A, B and C, to make an unique solution possible. The matrix was then solved by inversion using the University of Alberta's IBM 1620 electronic computor, utilizing a program based on Jordan's Method of Matrix Inversion (Jackson, 1962). The least squares

estimates were thus obtained in the form:

$$Y_{ijk..} = \hat{\mu} + \hat{a}_i + \hat{b}_j + \hat{c}_k + \ldots$$

where $\hat{\mu}$ = estimated population mean;

$\hat{a}_i$ = estimate of the effect of the i^{th} factor of the A classification, in the form $a_i - a_n$, where a_n is the estimate of the deleted equation;

$\hat{b}_j$ = estimate of the effect of the j^{th} factor of the B classification, in the form $b_j - b_n$, where b_n is the estimate of the deleted equation;

$\hat{c}_k$ = estimate of the effect of the k^{th} factor of the C classification, in the form $c_k - c_n$, where c_n is the estimate of the deleted equation.

The reduction in sums of squares due to fitting μ, a_i, b_j and c_k is $R(\mu,a,b,c,)$ and is given by:

$$R(\mu,a,b,c,) = \mu Y_{....} + \sum_i \hat{a}_i Y_{i...} + \sum_j \hat{b}_j Y_{.j..} + \sum_k \hat{c}_k Y_{..k.} + \ldots$$

The residual mean square which equals

$$\frac{1}{N - (m + n + p - 3)} \left[\sum_{ijk} Y_{ijk}^2 - R(\mu,a,b,c) \right]$$

is an unbiased estimate of σ^2.

In order to test for differences among the factors of the A classification, a second matrix was solved which was similar to the one described above but which excluded the A classification.

The resulting least squares estimates were used to calculate the reduction in sums of squares due to fitting u, b_j and c_k, which is $R(\mu,b,c)$ and is given by:

$$R(\mu,b,c) = \hat{u} Y_{....} + \sum_j \hat{b}_j Y_{.j..} + \sum_k \hat{c}_k Y_{..k.} + \ldots$$

The residual mean square which equals

$$\frac{1}{(m-1)} \Big[R(\mu,a,b,c) - R(\mu,b,c)$$

is an unbiased estimate of variance between treatments.

Similar matrices were solved in order to test for differences among factors of the remaining classifications for example B and C in this model.

Appendix Table 1

Subdivision of analysis of variance for general linear model using the method of fitting constants(1)

Due to	d.f.	Sums of Squares	Mean Square
(5) Fitting μ, a_i, b_j	m + n - 2	R (μ,a,b)	
Difference	p - 1	By Subt. (2) - (5)	C
(2) Fitting μ, a_i, b_j, c_k	m + n + p - 3	R (μ,a,b,c)	
(4) Fitting μ, a_i, c_k	m + p - 2	R (μ,a,c)	
Difference	n - 1	By Subt. (2) - (4)	B
(2) Fitting μ, a_i, b_j, c_k	m + n + p - 3	R (μ,a,b,c)	
(3) Fitting μ, b_j, c_k	n + p - 2	R (μ,b,c)	
Difference	m - 1	By Subt. (2) - (3)	A
(2) Fitting μ, a_i, b_j, c_k	m + n + p - 3	R (μ,a,b,c)	
Remainder	N...-(m + n + p - 3)	By Subt. (1) - (2)	E
(1) Total	N...	$\sum_{ijk} Y_{ijk}^2$	

(1) See test for definition of the symbols

The subdivision of the analysis of variance was as shown in Table 1. The ratios C/E, B/E, and A/E are compared with the F distribution with appropriate degrees of freedom

B. Analysis of data for 1961 calves

The method of least squares was used to estimate the effect of breeding group, sex and transferrin genotype on birth weight, calculated 180-day weight and calculated 365-day weight. Breeding group is defined as being made up of calves from dams of a given breed, source and age, and all sired by a given sire.

The mathematical model was organized as follows:

$$Y_{ijkl} = \mu + d_i + s_j + g_k + e_{ijkl}$$

where:

Y_{ijkl} is the value of the $ijkl^{th}$ calf for birth weight, calculated 180-day weight and calculated 365-day weight;

μ = the best estimate of the population mean;

d_i = the effect common to calves born in the i^{th} breeding group;

s_j = the effect common to calves of the j^{th} sex;

g_k = the effect common to calves of the k^{th} genotype;

e_{ijkl} = an effect particular to the l^{th} individual in the ijk^{th} group or a random effect due to error.

Based on the mathematical model above, twelve simultaneous equations were organized in the form of a 12 x 12 matrix with three right hand sides, for the data collected on the Hereford calves. Fourteen equations were similarly organized for the data collected on the calves from the hybrid herd. Three similar matrices were organized for each group of calves, each of which excluded one of breeding group, sex or genotype. Statistical treatment was as described above.

C. Analysis of data for 1962 calves

The method of least squares was used to estimate the effect of sire, breed group, sex and transferrin genotype on birth weight and calculated 180-day weight. Breed group is defined as being made up of dams of a given breed, source and age.

The mathematical model was organized as follows:

$$Y_{ijklm} = \mu + a_i + b_j + s_k + g_l + e_{ijklm}$$

where

Y_{ijklm} is the value of the $ijklm^{th}$ calf for birth weight and

calculated 180-day weight;

u = the best estimate of the population mean;

a_i = the effect common to calves sired by the i^{th} sire;

b_j = the effect common to calves born in the j^{th} breed group;

s_k = the effect common to calves of the k^{th} sex;

g_l = the effect common to calves of the l^{th} genotype;

e_{ijklm} = a random effect due to error.

Based on the mathematical model above, fourteen simultaneous equations were organized in the form of a 14 x 14 matrix with two right hand sides for the data collected on the Hereford calves. Twenty-two equations were similarily organized for the calves from the hybrid herd. Four similar matrices were organized for each group of calves, each of which excluded one of sire, breed group, sex or genotype. Statistical treatment was as described above.

D. Analysis of data for cows

The method of least squares was used to estimate the effect of breed group, transferrin genotype and pregnancy on the accumulated weight loss during the periods from August 29th, 1961; to February 1st, 1962, to April 6th, 1962, and to June 26th, 1962 and on standard cow weight. The latter was calculated as the average of a post weaning weight, October 25, 1961 and a post calving weight, June 27th, 1962. Breed group is defined as being made up of cows of a given breed, source and age.

The mathematical model was organized as follows:

$$Y_{ijkl} = \mu + b_i + g_j + p_k + e_{ijkl}$$

where:

Y_{ijkl} is the value of the $ijkl^{th}$ cow for accumulated weight loss for the periods described above and for standard cow weight;

u = the best estimate of the population mean;

b_i = the effect common to cows of the i^{th} breed group;

g_j = the effect common to cows of the j^{th} genotype;

p_k = the effect common to cows of the k^{th} pregnancy state;

e_{ijkl} = a random effect due to error.

Based on the mathematical model above, fourteen simultaneous equations were organized in the form of a 14 x 14 matrix with four right hand sides for the data collected for the Hereford cows. Fifteen equations were similarly organized for the cows from the hybrid herd. Three similar matrices were organized for the data for each group of cows, each of which excluded one of breed group, genotype or pregnancy. The statistical treatment was the same as described previously.

E. Analysis of data for 1962 calves for the effect of the cow's genotype on birth weight and calculated 180-day weight

1. Analysis of Hereford herd data

The method of least squares was used to estimate the effect of breed group, transferrin genotype and dehorning of the cows and the effects of sex of calf on birth weight and calculated 180-day weight. The definition of breed group is the same as that used above in the analysis of the cow data.

The mathematical model was organized as follows:

$$Y_{ijklm} = \mu + b_i + h_j + g_k + s_l + e_{ijklm}$$

where

Y_{ijklm} is the value of the $ijklm^{th}$ calf for birth weight and calculated 180-day weight;

μ = the best estimate of the population mean;

b_i = the effect common to calves born in the i^{th} breed group;

h_j = the effect common to calves from cows of the j^{th} dehorning;

g_k = the effect common to calves from cows of the k^{th} genotype;

s_l = the effect common to calves of the l^{th} sex.

e_{ijklm} = a random effect due to error

Based on the mathematical model above, thirteen simultaneous equations were organized in the form of a 13 x 13 matrix with two right hand sides. Four similar matrices were organized, each of which excluded one of breed group, dehorning, genotype or sex. The statistical treatment was the same as that described above.

2. Analysis of the hybrid herd data

The method of least squares was used to estimate the effect of breed group and transferrin genotype of the cow and the effect of sex of the calf on birth weight and calculated 180-day weight. The definition of breed group is the same as that used in the analysis of the cow data.

The mathematical model was organized as follows:

$$Y_{ijkl} = \mu + b_i + g_j + s_k + e_{ijkl}$$

where

Y_{ijkl} is the value of the $ijkl^{th}$ calf for birth weight and calculated 180-day weight;

μ = the best estimate of the population mean;

b_i = the effect common to calves born in the i^{th} breed group;

g_j = the effect common to calves from cows of the j^{th} genotype;

s_k = the effect common to calves of the k^{th} sex;

e_{ijkl} = a random effect due to error.

Based on the mathematical model above, fourteen simultaneous equations were organized in the form of a 14 x 14 matrix with two right hand sides. Three similar matrices were organized each of which excluded one of breed group, genotype or sex. The statistical treatment was the same as described previously.